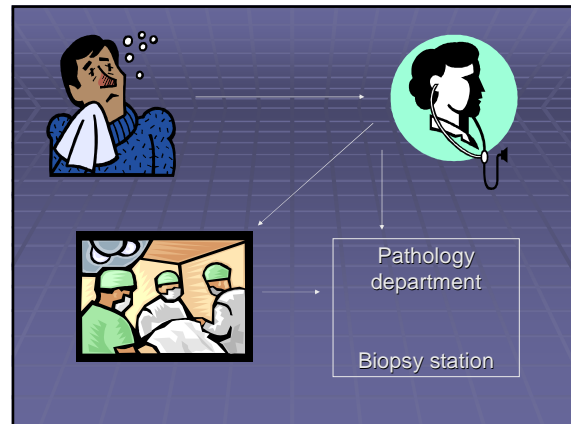
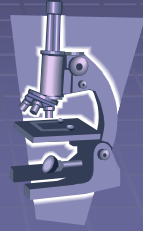


Methods in pathology. Basic histological techniques.

Prof. MUDr. Ján Jakubovský, DrSc.
RNDr. Martin Kopáni
MUDr. Peter Michalka

Prof. MUDr. Ľudovít Danihel, CSc.



- ✓ Material registration (biopsy station)
- ✓ Gross examination - Material sectioning
- ✓ Material processing:
 - ✓ Non fixed tissue (Touch imprint / smear preparation)
 - ✓ Fixation
 - ✓ Chemical (1 part of formol* : 9 parts of water
→ 10 % formol = 4 % formaldehyde)
 - ✓ Physical (freezing, cooking, microwave)
 - ✓ Embedding (parafine)
 - ✓ Cutting (microtome, cryostat)
 - ✓ Staining
 - ✓ Histological finding description
 - ✓ Diagnostical consideration
 - ✓ Diagnosis

* formol (formaline) = 40 % formaldehyde

Basic staining methods:

- ✓ Hematoxylin and eosin
- ✓ van Gieson
- ✓ Phosphotungstic-hematoxylin

Special methods:

Histological methods

Cytological methods

- ✓ gynecological (cervical smear)
 - ✓ Papanicolaou staining (polychromatic staining)
- ✓ Non-gynecological (sputum, body fluids, punctates, etc.)
 - ✓ HE
 - ✓ May-Grün-Wat-staining – Giemsa Romanovsky

evaluation by Bethesda (description),
before: Pap (1.-5. grade)

Chemical methods

- ✓ Histochemical methods
 - ✓ Protein-lipids-saccharids-lectin, metal histochemistry
 - ✓ Enzyme-histochemical methods
 - ✓ Immunohistochemical methods
- ✓ Fluorescent methods

Physical methods

- ✓ Transparent and incident light examination
- ✓ Polarized light microscopy (oxalate and other crystals, silicate elements, anizotropic lipids, and others)
- ✓ Electron microscopy
- ✓ Confocal laser scanning microscopy
- ✓ Historadiographic methods
- ✓ Phase contrast optical microscopy, Differential interference contrast microscopy
- ✓ Dark field optical microscopy

Basic staining methods

Tissue	Staining		
	HE	G	PtH
Chromatine	+	+	+
Cytoplasma	+	+	+
Basophilic plasma	+	+	+
Basophilic granules	+	+	+
Eosinophilic granules	+	+	+
Erythrocytes	+	+	+
Muscle	+	+	+
Collagen and hyaline	+	+	+
Fibrine and fibrinoid	+	+	+
Reticulin	+	+	+
Elastic fibres	+	+	+
Glia	+	+	+
Mucine	+	+	+
Lipids and cholesterol	-	-	-
Phospholipides	-	-	-
Glycogene	-	-	-
Calcium	-	-	-
Gram positive bacteria	+	+	+
Gram negative bacteria	+	+	+

Special chemical methods

Histological staining methods:

- ✓ **Elastic fibres** (Ezorcin and fuksin by Weigert)
- ✓ **Reticulin fibres** (Impregnation with the silver salts by Gomori)
- ✓ **Fibrin, fibrinoid** (Mallory phosphotungstic-hematoxyllin)
- ✓ **Thrombocytes** (Intense, shortly differentiated staining by Giemsa)
- ✓ **Lipids** (Sudan)
- ✓ **Mucin** (Mayers' mucikarmine)

- ✓ **Glycogen** (Best carmine)
- ✓ **Bacteria** (Gram, methylen blue, Giemsa staining)
- ✓ **Polysaccharides, neutral mucopolysaccharides, mucoproteins, glycoproteins, glycolipids** (PAS-reaction by McManus)
- ✓ **Acid mucopolysaccharides** (Hall method in modification by Müller or Gomori, alcian blue followed by metachromatic reaction)
- ✓ **DNA** (Feulger reaction)
- ✓ **Fe²⁺, Fe³⁺, Cu, Pb et.al.**

Enzymohistochemic methods:

slide → substrate → detection of reaction product

oxidase, peroxidase, dehydrogenase, phosphatase (alcalic, acidic)

Immunohistochemical methods:

Ag ↔ Ab

Direct method
Indirect method
Sandwich method

Peroxidase - antiperoxidase complex
Avidin - biotin complex (ABC)
In situ hybridisation
Histological slides processing - digestion

Fluorescent methods:

Fluorescence – transformation of invisible UV radiation to visible illumination - fluorochrome surface

- ✓ Own fluorescence (autofluorescence)
- ✓ Obtained fluorescence (staining of the tissue by fluorescent stains)

Electron and radioactive microscopic methods

- ✓ Raster - electron microscopy
- ✓ Transmission - electron microscopy
- ✓ SIMS analysis
- ✓ EDAX analysis