Lab Practical One

Week 1: Lab Methods and Organelles

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Background

- **Describe the steps in tissue preparation for microscopy:**
  - **Fixation**: typically the first step in the preparation of histological sections in where tissues samples are treated with fixatives as a means to preserve and protect from biological decay.
    - **Fixatives**: solutions, compounds, or others means meant to either disable degradative enzymes, induce cross-linking (stabilizing proteins), or protect from extrinsic damage.
  - **Embedding**: the process of placing tissues in a harder medium (e.g., paraffin and plastic resins) as a means to allow for thin slicing of tissue.
    - Embedding occurs later in the process of preparation; once a tissue is fixed it must first undergo a series of steps:
      - **Dehydration**: the removal of water using ethanol.
      - **Clearing**: replacement of an organic solvent miscible with both alcohol and the embedding medium, giving a translucent appearance.
      - **Infiltration**: evaporation of the clearing solvent via exposure to heat (50–60 °C) promoting the final embedding of tissue into the medium.
  - **Staining**: used as a means to increase contrast in tissue or specific features of tissue that are of interest as most biology tissue has very little inherent contrast.
    - Basophilic: dyes that have an affinity for anionic (net negative charge) cells parts.
      - E.g., hematoxylin, toluidine blue, alcian blue, and methylene blue.
    - Acidophilic: dyes that have an affinity for cationic (net positive charge) cell parts.
      - E.g., eosin, orange G, and acid fuchsin
- **What does H & E Stain?**
  - Hematoxylin (H) and eonsin (E) stains are among the most commonly used stains.
    - As mentioned above, hematoxylin acts as a basophilic dye, turning negatively charge organelles like the cell nucleus, RNA-rich regions of cytoplasm, cartilage, anywhere from blue → purple.
    - Eosin acts as an Acidophilic dye, typically turning cationic structures pink; sometimes it is considered to be a **counterstain**, i.e., typically a secondary dye that is meant to distinguish features.
- **What does PAS Stain?**
  - Periodic acid-Schiff (PAS) utilizes hexose rings of polysaccharides and other carbohydrate rich structures to stain macromolecules purple → magenta.

- **Describe Enzyme Histochemistry.**
  - Enzyme histochemistry is a method for localizing cellular structures using specific enzymatic activity in such structures.
  - Preservation of enzymes often requires non-fixed or mildly fixed tissue and generally adhere to the following steps:
    1. Tissues sections are immersed in solution containing the substrate of the enzyme to be localized.
    2. The enzyme is exposed to and allowed to act on the substrate.
    3. A marker compound is introduced and reacted with the product from step 2.
    4. Location is determined via precipitation of the insoluble product, which must be visible a light or electron microscopy, over the site of the enzyme.
  - Phosphatase, dehydrogenase, and peroxidase are common examples of enzymes detected with histochemistry.
- **How does Immunohistochemistry work?**
  - Immunohistochemistry (IHC): the use of labeled antibodies and antigens to identify and localize many proteins and macromolecules that lack specific enzymatic activity.
  - Visualization of such interactions are commonly accomplished with either:
    - Chromogenic immunohistochemistry (CIH): use of antibodies conjugated to an enzyme that catalzyes a color-producing reaction.
    - Immunofluorescence: tagging of a fluorophore (fluorescein, rhodamine) to an antibody.
  - Common used in diagnosis of abnormal cells such as those in cancerous tumors.

Microscopic Techniques

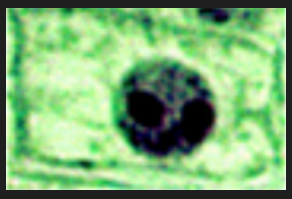
- **Bright field**:
- **Phase contrast**:
- **Confocal**:
- **Fluorescent**:
- **Scanning Electron Microscopy**:
- **Transmission Electron Microscopy**:

Organelles and Cytoplasmic Inclusions

Organelles and Cytoplasmic Inclusions

Structure	Size	Light Microscopic Features	Function
Nucleus	5–20 µm; largest organelle		
Nucleolus			
P. Membrane			
Rough ER			
Smooth ER			
Golgi Body			
Vesicles			
Mitochondria			
Endosomes			
Lysosomes			
Peroxisomes			
Cytoskeleton			
Ribosomes			
Glycogen			
L. Droplets			

Mitotic Phases

- 
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Apoptosis Events

- **DNA fragmentation**:
- **Decrease of cell volume**:
- **Membrane Blebbing**:
- **Formation of apoptotic bodies**:

Features and Functions

- **Stratified squamous epithelium**:
  - Features:
  - Functions:
- **Simple cuboidal epithelium**:
  - Features:
  - Functions:
- **Skeletal muscle**:
  - Features:
  - Functions:
- **Cardiac muscle**:
  - Features:
  - Functions:
- **Smooth muscle**:
  - Features:
  - Functions: