HISTOLOGICAL STAINS, AND SOME HINTS FOR ANALYZING SLIDES

1) STAINS

Many stains in histology were adapted from textile dyes; these stain tissues in the same way they dye cloth. Chemical reactions are also used to show up specific tissue components in special cases.

Dyes: salts - dissociate in aqueous solutions to form two ions
-one ion is colored, and can be either acidic or basic

Basic colored ions: bind with acidic components of cells, tissues
-these components are therefore called BASOPHILIC - they attract basic ions
-examples: nucleic acids in nuclei, ribosomes in cytoplasm

Acidic colored ions: bind with basic components of cells, tissues
-these components are ACIDOPHILIC - they attract acidic ions
-examples: cytoplasmic proteins are slightly basic, general cytoplasm stains with acidic dyes

Common dyes in histology:

Hematoxylin: oxidation of hematoxylin in solution produces a basic dye with a blue color
-stains BASOPHILIC parts of cells blue - nuclei, rough endoplasmic reticulum

Eosin: acidic dye, pink to red (color can be variable)
-stains ACIDOPHILIC parts of cell pink/red - proteins in cytoplasm and extracellular

Usually mixed together, hematoxylin and eosin (H and E) make the best general-purpose histological stain available, showing both cells and extracellular components:

CELL NUCLEI - stain darkly, usually blue/purple/black
CYTOPLASM and CELL STRUCTURES - stain red/pink; red blood cells stain bright red - if cells have dark red staining in cytoplasm, they are probably actively secreting acidophilic proteins
EXTRACELLULAR STRUCTURES - collagen or other fibres in connective tissue stain pink

Nissl stains: these are basic dyes, staining BASOPHILIC parts of cells - nuclei, ribosomes
-good for neurons, staining clumps of ribosomes associated with the rough endoplasmic reticulum in the neuron cell bodies

Trichrome stains: - Mallory's trichrome most common - these are dyes with multiple constituents mixed together, each dye staining a different structure

NUCLEI: black
CYTOPLASM, KERATIN, MUSCLE FIBRES: red/pink
CONNECTIVE TISSUE: blue - shows patterns of connective tissue among other tissues

Stains using chemical reactions:

Periodic Acid Schiff reaction: stains carbohydrates red to purple, shows locations of carbohydrate components in tissues, glycogen droplets in cell cytoplasm, basement membranes
-stains proteoglycans in basement membranes and in ground substance

Elastin stains: chemically react with elastin fibres in aorta, elastic cartilage and in connective tissue - blue/black

Reticular fibre stains: several types, stain Type III collagen blue/black

2) TISSUE RECOGNITION TECHNIQUES

The best way to learn histological material is to be able to analyze the form of the basic tissue elements present in a slide, and determine how these are related in the tissue or organ you are considering. Look at the slide by eye first, or look over the whole field at low power in the microscope or in any image you are presented with, and ask yourself a series of questions. What is the stain? The slide or image should identify this for you, but even if it is not identified it is usually hematoxylin and eosin. Can you identify any basic tissue types? How many tissue types are present? How are they related? Look at the cells; usually under medium to high magnification. Can you see nuclei? How many? This gives you a rough estimate of how many cells there are in the field of view.

If most of the field of view is full of cells and not much extracellular matrix, you are looking at a cellular tissue; is it epithelium? a gland? muscle? What are the cell types present? What is the size of these cells? Is their cytoplasm staining pale or dark? If the cytoplasm is relatively dark-staining, you may be looking at cells that are secreting protein. Are the nuclei large or small? centered in the cytoplasm or off-center? Is there a nucleolus? How do the different cell types present relate to each other?

If there are relatively few cells and more extracellular matrix, is this connective tissue? Do you see any blood vessels or other features? How are elements in the matrix stained? Are there fibres, and how are they arranged? No fibres?

In this process, you are trying to determine the pattern of cells and extracellular elements in the slide you are looking at; once you have analyzed the slide this way, you can then work out what organ or structure is likely to have this pattern and what possibilities can be eliminated.

3) THREE-DIMENSIONAL STRUCTURES FROM TWO-DIMENSIONAL SECTIONS

One of the most difficult tasks in learning histology is determining the three-dimensional structure of a tissue or organ from a thin section taken from that structure. Looking at the slide

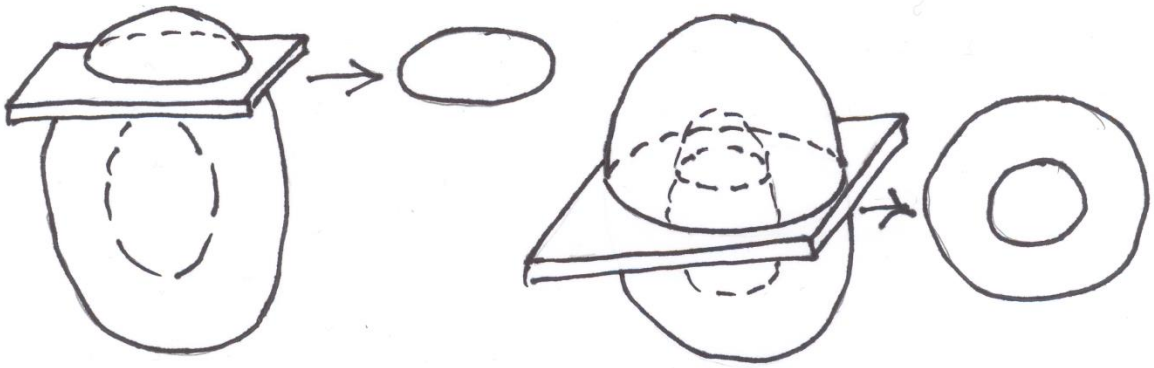
by eye or under low power, it may be possible to determine how the section was cut; for instance, if you know that the section is from a blood vessel, you know that it is a tube and you can then figure out how the section was oriented.

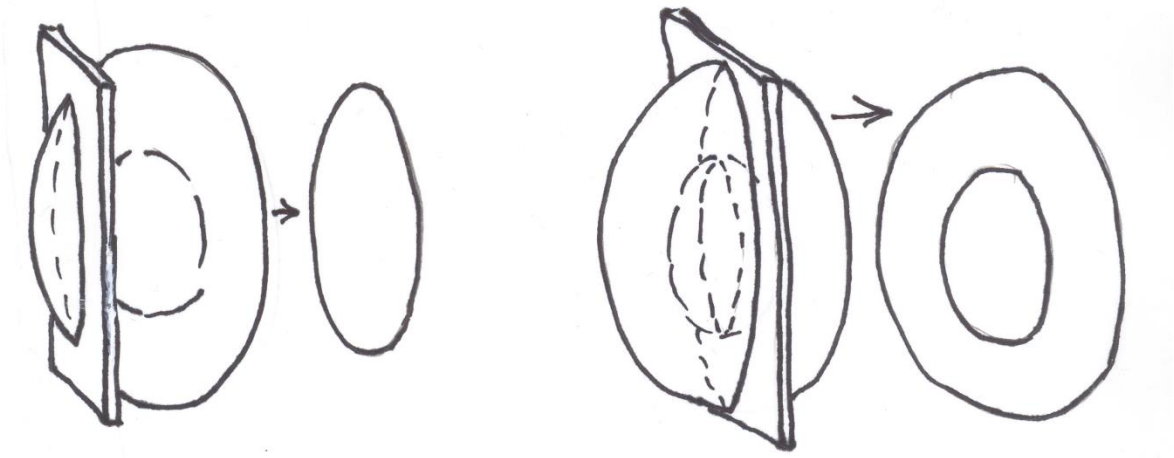
This process can be simplified. Most parts of the body contain only 5 types of structures at the histological level:

round or oval structures: cells, acini, roughly sphere-shaped organs
tubes: hollow, straight, curved or coiled
sheets of cells or tissue: flat or curved
partitioned structures: structures that have internal divisions usually marked by connective tissue septae
cord-like structures: long, usually round in cross-section, with solid things inside - could be nerves, muscles

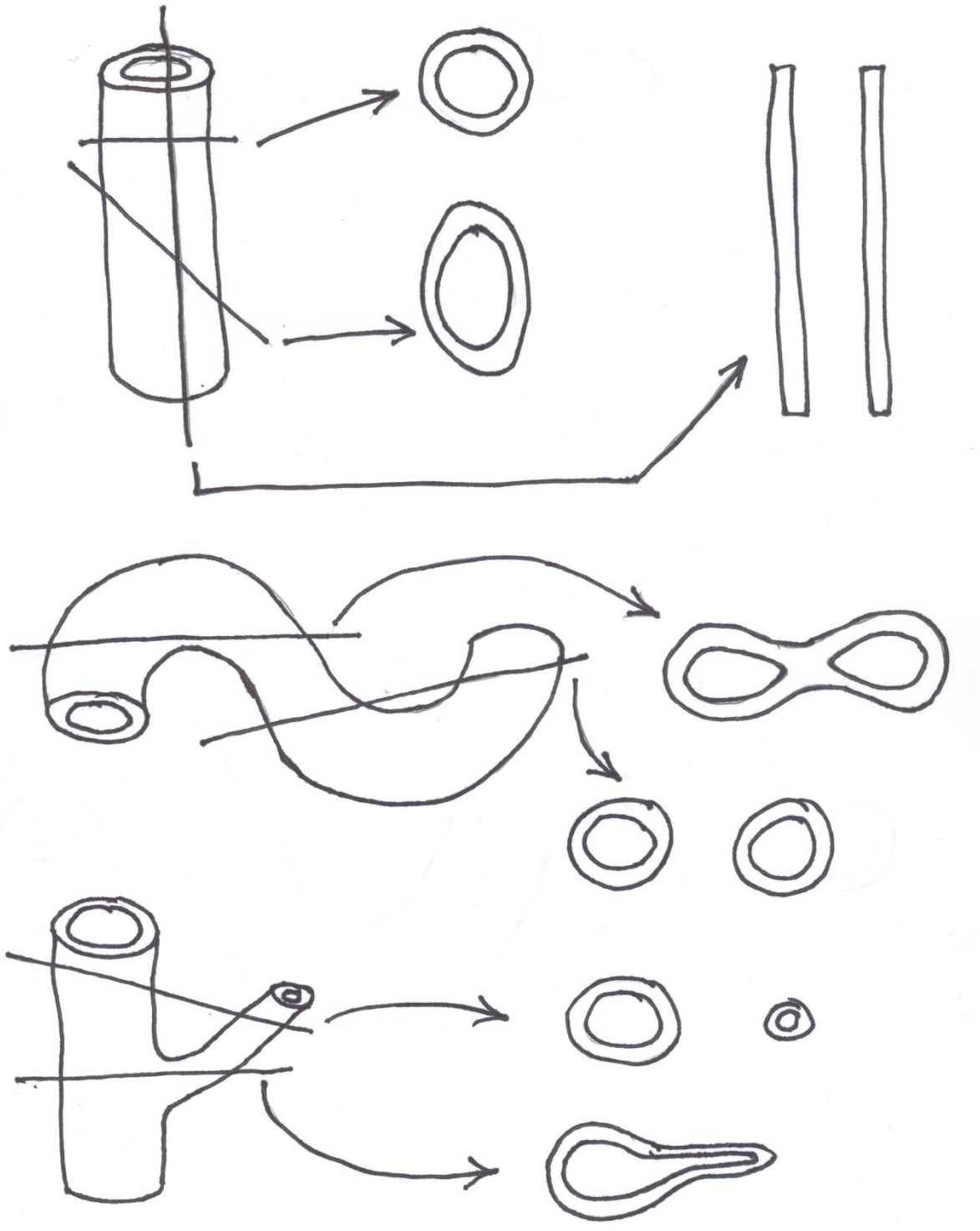
In order to help you interpret the shapes of these structures in histological sections, the following diagrams show what typical sections of these might look like when cut at different angles.

SECTIONING ROUND OR OVAL STRUCTURES

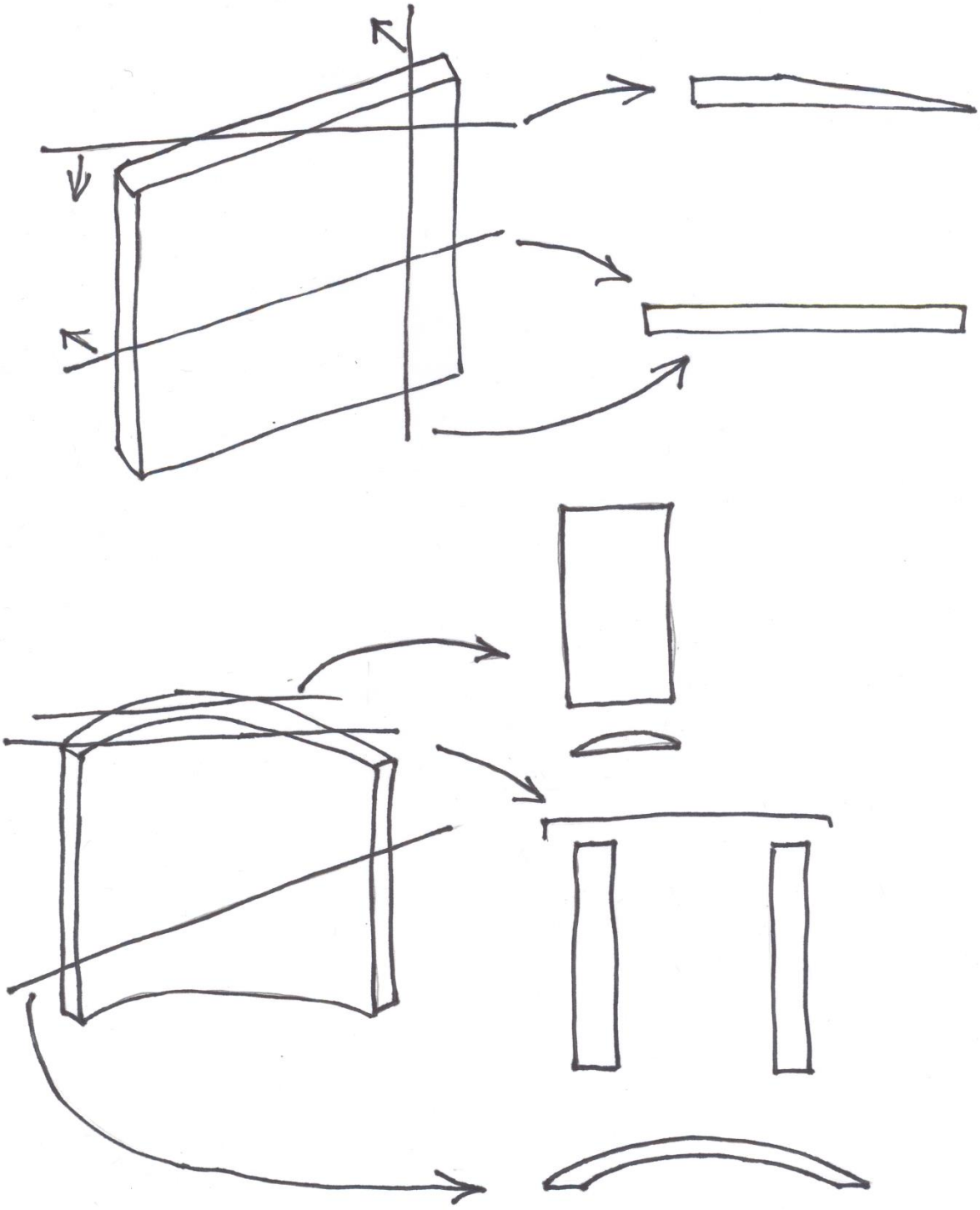




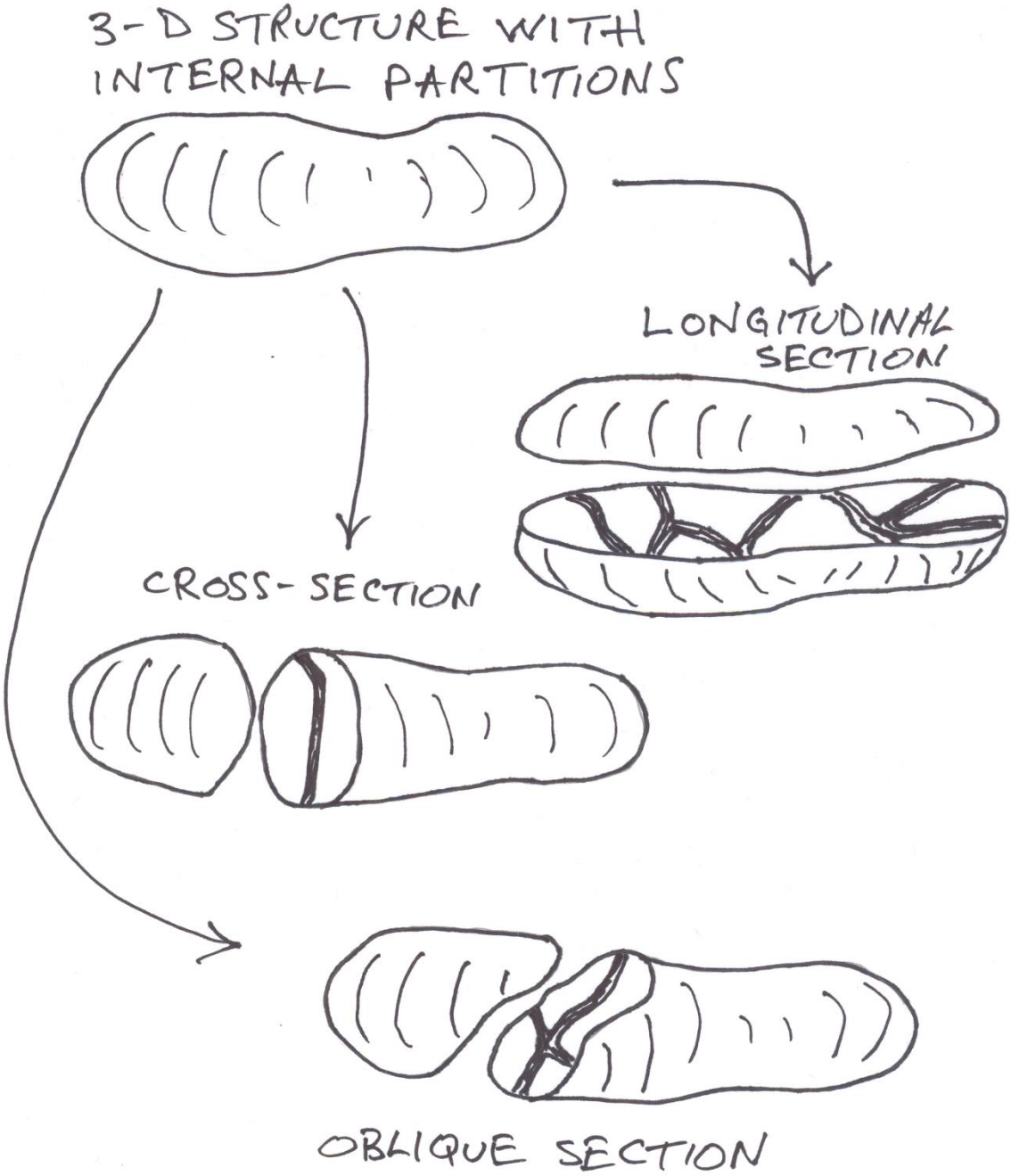
SECTIONS OF TUBES



SECTIONING SHEET-LIKE STRUCTURES



SECTIONS OF PARTITIONED STRUCTURES



SECTIONS OF CORD-LIKE STRUCTURES