LABORATORY 2 - THE CELL, continued

This lab has an associated homework assignment that is posted on Blackboard. If you have not done this assignment already, it would make this lab more efficient if you did so.

OBJECTIVES: Today in addition to the objectives stated previously, you will begin to analyze electron micrographs. The lab manual instructions will direct you to observe cell organelles and other cell components in electron micrographs and in glass slides.

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

SL181 (spinal cord) rough endoplasmic reticulum
SL108 (pancreas) vesicles
SL 29 (whitefish embryos) bundles of microtubules
SL 7A (liver, normal and fasted) glycogen stain
SL 7B (liver, normal) glycogen stain, predigested with amylase
SL 8 (fat) fat
SL 9 (skin) pigment

ELECTRON MICROGRAPHS (Gray envelope and text)

EM 3-6, 4-5, 12-1, 16 plasma membrane
EM 1-3, 2-1, 2-2, 3-5, 4-1, 10-5, 14-3 rough endoplasmic reticulum
EM 4-2, 10-6, 12-3, 13-7, 14-5 smooth endoplasmic reticulum
EM 5-2 and 5-inset ribosomes
EM 1-1 to 11-4 Golgi apparatus
EM 6-10 to 6-13, 2-3 to 2-5 also 3-4, 1-4, 12-2 and 13-6 mitochondria
EM 1-10, 4-3, 13-2 and 14-1 lysosomes
EM 1-5 peroxisomes
EM 4-6 and 4-7 microtubules
EM 4-4, 10-2 microfilaments
EM 1-7, 5-3, 13-1 & 5, 14-4 Glycogen
EM 10-4 Lipid
EM 3, 4, 16 and 17 Magnification and Resolution

EM = Electron micrograph from gray envelope in desk drawer

Electron micrograph in gray envelope in desk drawer (e.g. EM 20-2 means item or area designated 2 in electron micrograph #20)

POSTED ELECTRON MICROGRAPHS

1 Organelles
6 Organelles
Lab 2 Posted EMs

RELATIONSHIP OF MAGNIFICATION AND RESOLUTION IN ELECTRON MICROGRAPHS

CONTINUED, NEXT PAGE

HISTOLOGY IMAGE REVIEW - available on computers in HSL

Chapter, 3, Cytology
Frames: 88-126

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., An Atlas of Histology
Copies of this text are on reserve in the HSL.
A digitized version of the text can be found at: http://projects.galter.northwestern.edu/rhodin/

Plasma membrane	Fig. 2-2; 2-3
Rough ER	Fig 2-26; 2-29; 2-30; 2-31; 2-32
Smooth ER	Fig 2-33; 2-34; 2-35
Ribosomes	Fig 2-27; 2-28; 2-30; 2-31; 2-32
Golgi apparatus	Fig 2-36; 2-37; 2-38
Mitochondria	Fig 2-39; 2-40; 2-41
Lysosomes	Fig 2-46; 2-47; 2-48; 2-49; 2-50; 2-50; 2-52; 2-53; 2-54
Peroxisomes	Fig 2-55
Microtubules	Fig 2-62; 2-63; 2-64
Intermediate filaments	Fig 2-68
Glycogen	Fig 2-33; 2-78
Lipid	Fig 2-74

(Detailed instructions for this lab appear on the following pages.)

II. THE CELL, CONTINUED - LIGHT AND ELECTRON MICROSCOPY

In today's laboratory you will begin to study electron micrographs. For each new structure studied today both light microscopy (section A) and electron microscopy (section B) will be considered. In addition, the organelles studied in the previous laboratory session will be studied by electron microscopy. The light microscopic section is designed to demonstrate structural features that can be visualized by routine and special staining techniques. You should be aware that there are many staining methods for special purposes, but in the slides we will use, H and E and PAS are the more important stains. The electron micrographs demonstrate the same structures observed with the light microscope, at the "ultrastructural" level of organization. Compare the two methods of study and in every instance relate the structure to its contribution or role in the normal function of a cell or organ. It is important for you to retain the approach of a cell biologist throughout the course as you study different tissues and different organs.

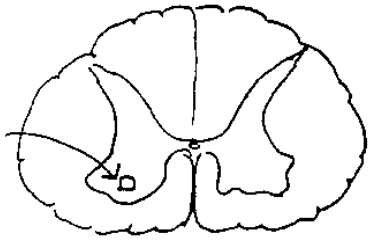
1. PLASMALEMMA OR PLASMA MEMBRANE - The plasma membrane forms the outer boundary of cells and consists of trilaminar, unit membrane. Prior to considering other organelles by electron microscopy you should become familiar with the appearance of the plasma membrane.

Electron Microscope - Study diagrammatic representation in text and atlas (J. Figs. 2-1 to 2-8; R. Figs. 2.2 to 2.11). Observe the plasma membrane in EM 3-6, 4-5, 12-1. Note that cells have many membranes, the outermost membrane is the cell membrane or plasma membrane (or plasmalemma). In EM 16 locate the plasmalemma and note its trilaminar (three-layered) appearance (J. Fig. 2-1; R. Fig. 21.2). Remember that all plasma membranes and all membranes in the cell appear trilaminar since they have two dark laminae separated by a central light lamina *when sufficiently magnified*.

ENDOPLASMIC RETICULUM. Endoplasmic reticulum is a membranous organelle that is formed of flattened cisternae or irregular tubules. A variety of enzymes may be associated with the ER. Two distinct types of ER are found and are often interconnected, rough ER (RER), that has ribosomes associated with it and smooth ER (SER), in which the organization of membranes is different and ribosomes are absent.

2. ROUGH ENDOPLASMIC RETICULUM

A. Light Microscope (SL 181) (scan, low) - Rough surfaced endoplasmic reticulum cannot be seen, as such, with light microscopy but in cells in which it is abundant, its presence can be detected and its intracellular distribution determined by staining for RNA. The RNA is the structural RNA (rRNA) associated with the ribosomes that line the surface of the RER. In these cases it is known as cytoplasmic basophilia or ergastoplasm as seen in nerve cells (J. Fig. 9-3; R. Plate 31) or neurons (SL 181). The neurons are located in the region shown in the rectangle on the left side of the diagram below (enclosed by red line). In this particular instance, the RER is stained by a stain called Nissl stain and is referred to as Nissl substance (red arrows) (J. Fig. 9-3; R. Plate 31). Neurons have extensive RER in the cytoplasm that appears as irregularly shaped masses



B. Electron Microscope - (J. Figs. 2-16, 2-21; R. Fig. 2.25, 2.26, 2.30). Observe RER in electron micrographs. EM 1-3, low magnification 2-1 (cisternae), 2-2 (ribosome). Also note in EM 3-5, 4-1, 10-5, and 14-3. What is the function of RER?

3. SMOOTH ENDOPLASMIC RETICULUM

A. Light Microscope – Smooth endoplasmic reticulum is not specifically distinguishable by light microscopy.

B. Electron Microscope (J. Fig. 20-13; R. Fig. 2.31). Observe series of membranes of SER. EM 12-3, 10-6, 13-7, and 14-5. Note in EM 4-2 the continuity of SER membranes with those of RER. Functions?

4. RIBOSOMES

Electron Microscope (J. Fig. 2-14, 2-16; R. Fig. 2.30). Single ribosomes or multiple ribosomes linked together by mRNA to form polysomes (polyribosomes) may be found either free in the cytoplasm or associated with a membrane system, the rough endoplasmic reticulum. EM 5-2 shows cytoplasm that contains ribosomes and polyribosomes, compare the size of ribosomes to other cell constituents. In EM 5 (inset) observe that polysomes are formed of evenly spaced ribosomes. These ribosomes are linked together with a strand of messenger RNA, but the mRNA is not visible in the micrograph. What is the function of polysomes?

5. GOLGI APPARATUS

Electron Microscope - (J. Fig. 2-20, 2-21, 2-22; R. Fig. 2.33). Identify the Golgi apparatus in EM 11 note 11-1, transport vesicles; 11-2 flattened cisternae of the cis (forming) face; 11-3 dilated cisternae of the trans (maturing) face; and 11-4 secretory vesicles. Function?

6. MITOCHONDRIA

Electron Microscope - (J. Fig. 2-12; R. Fig. 2.37). Note the detailed structure of a mitochondrion EM 6-10 (matrix), 6-11 (cristae), 6-12 (outer mitochondrial membrane) and 6-13 (granules); 2-3 (mitochondrial cristae), 2-4 (outer mitochondrial membrane), and 2-5 (inner mitochondrial membrane). Also note mitochondria in EM 1-4, 3-4, 12-2, and 13-6. Review functions of mitochondria and the locations of these functions.

7. LYSOSOMES

Electron Microscope - Lysosomes may be found in almost every cell during some period of the cell's life (J. Figs. 2-24, 2-26; R. Fig. 2.23). Newly made lysosomes bud from the Golgi as primary lysosomes EM 1-10, and are typically small, dark, and homogenously stained. After fusion with another vesicle, these become secondary lysosomes 4-3, 13-2, and 14-1, which are heterogeneously stained and vary considerably in size.
Note – Depending on their function and/or stage, secondary lysosomes may be referred to as residual bodies, autophagosomes, phagolysosomes, or multivesicular bodies. You need not distinguish these subtypes of secondary lysosomes in the laboratory (simply call them secondary lysosomes).

8. VESICLES

Light Microscope SL 108 Pancreas (R 2.12) (scan, low, med). Near the center of each of the numerous round structures in this section a pink region is evident. Careful observation reveals that this eosinophilic region is composed of many very small round structures that are secretory vesicles (secretory granules) (within blue circles). Vesicles are small membrane bound

"cavities" (these secretory vesicles contain proteins) and are associated with a variety of functions within a cell. Their significance will be described as they are encountered in specific cell types. Generally, individual vesicles are not visible with the light microscope. (Why not?)

FYI: Toward the right of this slide, there are several sections of blood vessels (yellow stars). Some of these vessels are filled with a number of disk-shaped red blood cells (note that some vessels are empty). We will study blood cells and vessels later in the course.

Electron Microscope - (J. Fig. 2-23, R.18.22). Observe secretory granules in EM 3-1.

9. PEROXISOMES

Peroxisomes (J. Fig. 2-27; R. 18.12, EM 1-5) are visible in the electron microscope, they are similar in size to primary lysosomes and contain the enzymes catalase and oxidase. Functions?

CYTOSKELETON - Virtually all cells contain the filamentous structures that are included in the category of cytoskeleton. Cytoskeletal elements include microtubules, microfilaments and intermediate filaments.

10. MICROTUBULES – (J. Fig. 2-29; R. Fig. 2-39) These tubular structures have a diameter of about 24 nm. They occur as single structures and also form the supportive element of cilia and flagella.

A. Light Microscope – The diameter of a microtubule is too small for it to be seen in the ordinary light microscope, but when microtubules occur in groups or are specifically labeled with antibodies, they may be detected. Also, special silver stains allow microtubules and other elements of the cytoskeleton to be visualized in the light microscope.

(SL 29) Bundles of microtubules - In this slide of whitefish embryos many cells are in mitosis. Mitotic spindles (J. Fig. 3-15, 3-16; R. Fig. 2.39, 3.14 to 3.16) in these cells are composed of a large number of closely associated microtubules. The aggregated microtubules are evident in the light microscope (red arrows). The spindle fibers form arcs that extend between the poles of the cells.

B. Electron Microscope - (J. Figs. 2-29 to 2-31, 3-16; R. 2.39). Locate microtubules in EM 4-6, 4-7. Study their distribution and function in the text.

11. CENTRIOLES Centrioles are located near the nucleus and participate in cell division. Study these in your text. (J. Fig. 2-31, 2-32; R. Fig. 2.55).

12. MICROFILAMENTS - Composed of actin, microfilaments occur in most, if not all, mammalian cells and are regarded as components of the cytoskeleton.

A. Light Microscope – (R. Fig. 2.45) Microfilaments are not visible in routine preparations, but aggregates may be demonstrated by a variety of immunolabeling techniques. The diameter of microfilaments is 7 nm, smaller than the other filamentous components of the cytoskeleton. Microfilaments are composed of the protein actin and usually play a role in contractility.

B. Electron Microscope - (J. Fig. 2-29;). Observe examples in EM 4-4 and 10-2. Study distribution and function in the text. Note a more detailed study will be made in subsequent sections of the course, e.g., with muscle.

13. INTERMEDIATE FILAMENTS (J. Fig. 2-34; R. Fig. 2.48, 2.50). Intermediate filaments are also components of the cytoskeleton. All intermediate filaments are about 10 nm in diameter and

appear similar in electron micrographs; however, their protein subunits are tissue specific. They will be described later when tissues are considered in which they are a special component. These filaments are numerous in many types of cells. They function in a structural capacity and may participate in other functions as well.

INCLUSIONS. In routine H and E stained sections many inclusions often are not apparent. In H & E vacuolated cytoplasm might be the only suggestion of the inclusion. What is the functional significance of each of the following inclusions?

1. GLYCOGEN

A. Light Microscope. SL 7A and SL 7B - Rat liver stained for glycogen (PAS and hematoxylin). There are three sections of tissue on this slide; 1) normal liver; 2) normal liver section digested with amylase; glycogen is digested by this enzyme and its removal from this section indicates that the stained substance was glycogen, and 3) liver from a rat fasted 4 days (physiologic reduction of glycogen). Most slides will have two sections at one end (SL 7A) and a single section at the other (SL 7B). The section that is most intensely red is normal; the single section is the digested one. Note the numerous red-stained granules in the cytoplasm of the normal (blue circles) cells. In contrast, in the digested section (blue circles), red granules are absent. Compare cell size, glycogen content and vacuolization of the cytoplasm and describe the differences in the appearance of the digested section and the section from the fasted rat (blue circles)?

B. Electron Microscope. (J. Fig. 2-35b; R. Fig. 2.58). Identify glycogen in EM 1-7, 5-3 (compare to size of ribosomes), 13-1, 13-5 and 14-4.

2. LIPID NEUTRAL FAT

A. Light Microscope - SL 8 - Hamster brown fat (Osmic acid). Note the black droplets of various sizes in the cells. Cell outlines are not distinct. Some slides have cells with a single large lipid mass. Fat, as a tissue will be studied in a later section (scan, med, high, within red circle).

B. Electron Microscope - Identify lipid droplets (J. Fig. 2-35a; R. 9.3) in EM 10-4.

3. **PIGMENT.** Pigment granules are usually membrane-bound and are regarded as organelles by some authors and as inclusions by others.

A. Light Microscope - SL 9 – Pigmented skin (H and E). (J. Fig. 2-40). Several layers of cells cover the entire surface of this structure (red arrows). The deepest layer is a row of dark, more heavily pigmented columnar cells. Locate scattered granules of pigment (melanin) in many of the other cells. This is one of several pigments, endogenous as well as exogenous, encountered in the body. The normal distribution of pigments in tissue sections, as well as gross appearance, is important. Special note should always be made of alterations in normal patterns as these may be of clinical significance. Other pigmented tissues will be encountered in subsequent studies (low, medium, high, red arrows).

B. Electron Microscope – Pigment granules are visible as small osmiophilic granules in many cells EM 20-5.

RELATIONSHIP OF MAGNIFICATION AND RESOLUTION IN ELECTRON MICROGRAPHS

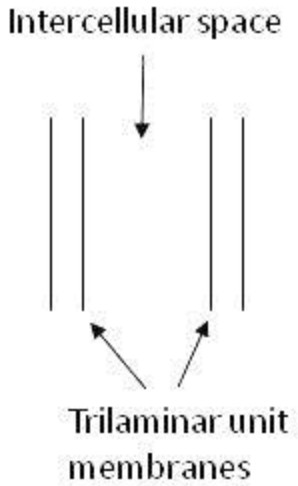
The relationship of magnification and resolution in the evaluation of electron micrographs (EM) # 17, 3, 4, 16 (in gray envelope).

EM 17 — Notice that the image includes about four "whole" liver cells and parts of adjacent cells. Even if there were no micron (micrometer) marker on the micrograph, it is evident that this is a low magnification image. Cell boundaries are visible because there is a slight separation (extracellular space) between the cells, but, because the magnification is low, a definitive plasma membrane is difficult to see in this image, and intracellular details such as ribosomes and ER are hard to discern. The micrometer marker indicates the magnification in the print is about 5,500 X.

EM 3 — This micrograph is an image of pancreatic acinar cells; one "whole" cell and portions of two adjacent cells are included in the field. If no micron (micrometer) marker were on the micrograph, it would be reasonable to conclude that this image is a higher magnification than #17 because fewer cells are in the field, and intracellular structures are more easily seen in #3. Notice that the item marked 6 and identified, as plasmalemma in the micrograph appears to be a single line. At this magnification a single line is in fact the closely apposed parallel plasma membranes of two adjacent cells. The cell's basal surface is readily detected because of the presence of a basal lamina (7). The micron marker indicates magnification of the print is about 10,000 X.

EM 4 — The identification of this portion of a cell as an intestinal absorptive cell is based on the knowledge of the source of the image, since characteristics typical of an absorptive cell (apical surface with microvilli) are not included in the field. Many other cell types have the same organelles as shown here. Without the micron (micrometer) marker, it is apparent that this image was photographed at a higher magnification because only a small portion of a cell occupies most of the field, with only small portions of adjacent cells (upper left corner, lower right corner). The mitochondria are larger, their double membrane apparent, and ribosomes are easily identified. The parallel plasma membranes of adjacent cells, item 5, are clearly separated from each other by a small intercellular space. The cell membranes in the lower right corner "disappear" in the image in areas where they are not cut exactly transversely by the plane of section; the membranes in the upper left corner are more transversely cut, thus more distinct over a greater distance. The micron (micrometer) marker indicates that this image is magnified about 66,000 X.

EM 16 — Without the micron (micrometer) marker this image would be determined to be very high magnification because only a small portion of the apical surface of two adjacent cells is shown. No mitochondria are present and most other cell organelles are excluded from this limited apical region. The apical surface with microvilli is evident as well as the junctional complex that includes a zonula occludens (1), a zonula adherens (2), and macula adherens (desmosomes) (3). In ideal transverse planes of section through the plasma membranes, the trilaminar unit membrane is visible. The closely parallel plasma membranes can be viewed as two trilaminar membranes (dark-light-dark) on both sides of a narrow (wide at this magnification!) intercellular space. The micrometer marker on this image indicates the magnification is about 120,000 X.



Compare this image in EM 16 with the appearance of two parallel plasma membranes in EM 4 (also dark-light-dark) but not at high enough magnification to reveal the trilaminar unit membranes.

OBJECTIVES FOR LABORATORY 2: THE CELL II

1. Using the light microscope or digital slides, identify:

   Endoplasmic reticulum – when stained with Nissl substance in neurons
   Secretory vesicles – note general location in apical region of cytoplasm
   Microtubules – only visible as bundles (e.g. mitotic spindle)
   Glycogen – when stained with PAS/hematoxylin
   Fat – when using special stains (e.g. osmic acid)
   Pigment

2. On electron micrographs, identify:

   - Plasma membrane
   - Endoplasmic reticulum
     - Smooth
     - Rough
       - Cisterna
       - Ribosome
   - Free ribosomes
     - Polysomes / polyribosomes
   - Golgi apparatus
     - Transport vesicles
     - Cis face
     - Trans face
     - Secretory vesicles
   - Mitochondria
     - Matrix
     - Cristae
     - Outer mitochondrial membrane
     - Inner mitochondrial membrane
     - Inter-membrane space
   - Lysosomes
     - Primary lysosomes
     - Secondary lysosomes
   - Secretory vesicles
   - Peroxisomes
   - Cytoskeletal elements
     - Microtubules
       - Centrioles
     - Microfilaments
     - Intermediate filaments
   - Inclusions
     - Glycogen
     - Lipid droplets
     - Pigment