Contents

Helpful Hint

The higher-magnification lenses have longer tubes on the turret and, therefore, sit closer to the glass slides. Using the coarse focus knob with high-magnification lenses has the risk of driving the lens through and breaking the slide or, worse, damaging the objective lens.

Finally, the eyepieces, or oculars, also magnify the image (usually 10X). It is beneficial to use both eyes when looking at a specimen; this will save a lot of headaches. One important adjustment to the eyepieces is the interpupillary distance, which can be adjusted on the microscope to match the user's eyes. Note that some microscopes have an **interpupillary adjustment roller**, while others allow free movement of the eyepieces.

Another important adjustment of the eyepieces ensures that both eyes see the image in focus. One of the eyepieces has a **diopter adjustment ring**. This changes the focal plane for that eyepiece. To make this adjustment, start by closing the eye corresponding to the adjustable eyepiece (the left eye on the microscope in **Fig. 1.1**) and adjust the focus for the other eye (the "fixed" eyepiece) using the coarse and fine focus knobs until the image is sharp through that eyepiece. Then close that eye and adjust the focus on the other eyepiece with the diopter adjustment ring until the image is sharp through that eye as well.

1.1.2 Digital Microscopy

Traditional microscopes have been used for centuries to look at samples on glass slides and are still used today by many practitioners and researchers. However, digital slide technology is becoming more common and is the format that many schools utilize to study histologic specimens.

To create digital slides, glass slides are scanned in a slide scanner. There are several formats for the resulting data, but ultimately digital slides can be viewed using any computer (**Fig. 1.2**). Mouse controls are fairly intuitive. For example, dragging and dropping is used to move around the slide. The mouse may have a scroll wheel that can be used (sometimes with the Ctrl key) to change magnification, or magnification can be selected from a menu (**Fig. 1.2**, circle). The inset in the upper right shows a scanning view of the slide. The box in the inset shows the user where the view is on the slide, and it changes size depending on magnification. As mentioned, each digital slide platform is different, and functionality varies based on operating system, type of mouse, and other features of the computer system. The best way to learn is by experimentation.

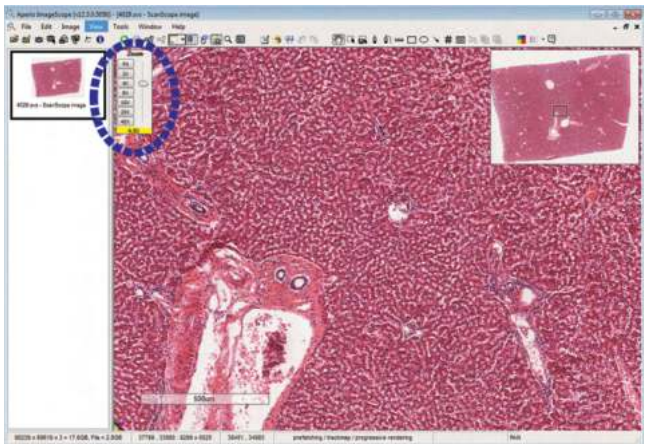

Fig. 1.2 **Digital slide.**

1.1.3 Traditional versus Digital Microscopy

When using a traditional light microscope, magnification is determined by multiplying the magnifications of the objective and ocular lenses. The magnification of the objective lens is selected by the user, while the eyepiece magnification is typically 10X. So, with the nosepiece set to the 40X objective, then the total magnification of the image is 400X (40 × 10).

When creating digital slides, only the objective lenses equivalent to the ones on a traditional microscope are used. Depending on the system, the digital slide view may include a zoom bar that shows the magnification of the image. The magnification can be changed by adjusting the cursor on the magnification bar or by zooming in and out using the mouse scroll wheel. Typically, the magnification bar goes only to 20X or 40X, so one would naturally think that the magnification of digital slides pales in comparison to traditional microscopes. However, remember that the traditional microscope image is projected through tiny eyepieces, while digital slides are projected on large computer screens at high resolution.

Helpful Hint

To approximate magnification on digital slides, users typically multiply the magnification in the zoom bar by 10. This isn't exact but close enough, and it compares quite well to what is seen in the traditional light microscope. In this publication, scale bars will be included in images as often as possible.

1.1.4 Other Types of Microscopy

There are other types of microscopy used to visualize cells and tissues (e.g., phase contrast, confocal), which are beyond the scope of this publication. One that needs to be mentioned here is transmission electron microscopy. The basic principles of transmission electron microscopy are quite similar to those of the light microscope, except that electrons are passed through a specimen instead of light. This provides greater magnification and resolution than light microscopy. The study of electron micrographs will be addressed in a subsequent chapter.

1.2 Basic Features of Cells and Tissues

Before delving into detailed histology, it's a good idea to start with a simple drawing of a single cell (**Fig. 1.3**). Although this book will not cover detailed cell biology (e.g., at the molecular level), the general structure and function of the cell's components will be described in subsequent chapters. For now, appreciate that:

- The outer membrane of the cell, the **plasma membrane**, separates the cell's contents from the extracellular matrix.
- The cell contains many **organelles**, of which the most prominent is the **nucleus**.
- The content of the cell other than the nucleus is referred to as the **cytoplasm** (or **cytosol**, though the two are not exactly the same).

Basic Science Correlate

Technically, the cytoplasm is everything in the cell other than the nucleus, whereas the cytosol is the liquid component of the cytoplasm—that is, the cytoplasm minus the organelles seen in the image (e.g., mitochondria, endoplasmic reticulum). However, these two terms are often used interchangeably.

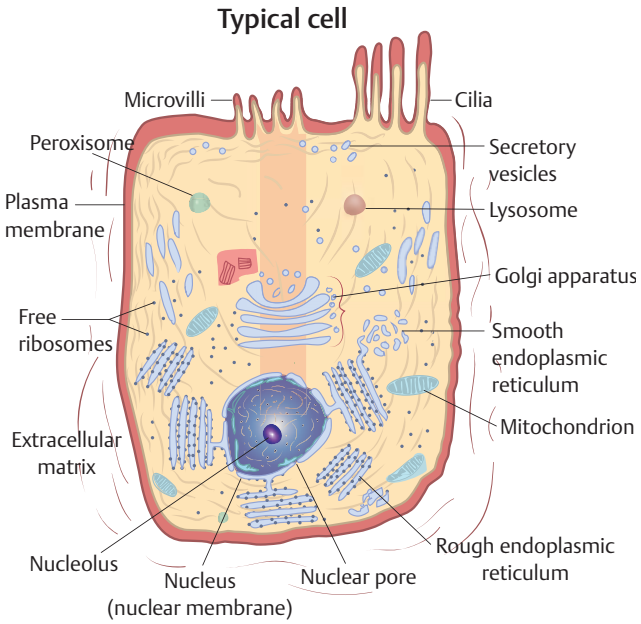

Fig. 1.3 **Simplified diagram of a typical cell.**

Fig. 1.4 is an image taken from a slide of the liver. The liver is a good place to start because the cells are clearly defined (one cell is outlined in green).

The most prominent organelle in the cell, and the only one easily seen in the light microscope, is the nucleus (**Fig. 1.4**, blue arrows), which appears blue on this slide. The remainder of the cell (pinkish-purple on this slide) is the cytoplasm; the organelles within the cytoplasm are too small to visualize at this resolution.

The material between the cells is the extracellular matrix. However, this is complicated somewhat in the liver (and other organs), since the paler pink tissue between the cells includes blood vessels, which are composed of some cells, and other connective tissue cells (more on this later).

Helpful Hint

There are some cells with two nuclei; this is not unusual for liver and other types of cells.

Video 1.1 Cells, nuclei, cytoplasm, and extracellular matrix

Be able to identify:
- Cells
  - Nuclei
  - Cytosol (cytoplasm)
- Extracellular matrix

https://www.thieme.de/de/q.htm?p=opn/tp/308390101/978-1-62623-414-7_c001_v001&t=video

In the cells shown in **Fig. 1.4**, it is easy to pick out cell borders. However, this is the exception rather than the rule. The strip of tissue between the green brackets in **Fig. 1.5** contains approximately 30 to 50 cells, based on the number of nuclei in that region (one nucleus bounded by blue arrows). The pink region

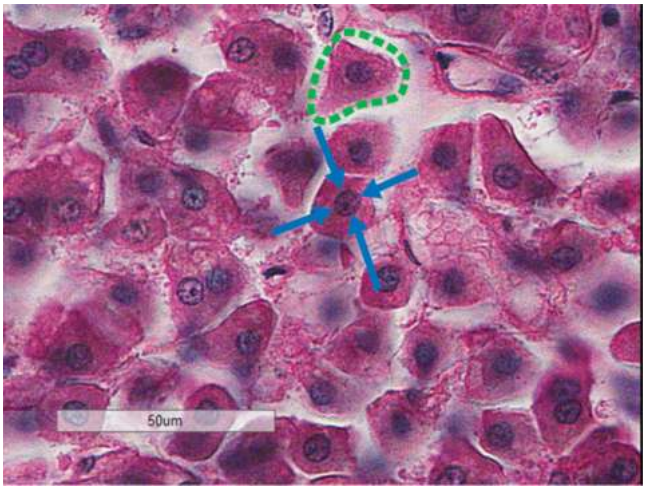

Fig. 1.4 **Liver, indicating cells (green outline) and nuclei (blue arrows).**

around, but mostly above, the nucleus is the cytoplasm. The plasma membranes between the cells are, for the most part, not visible, though they are visible in some locations (black arrows).

Helpful Hint

When cell borders are not obvious, it helps to draw them in mentally to get a sense of the shape and size of the cells. For example, the mentally drawn outline of a single cell (**Fig. 1.5**, black dotted outline) indicates that these cells are columnar in shape (rectangular).

Video 1.2 Cells, nuclei, cytoplasm, and extracellular matrix

Be able to identify:
- Cells
  - Nuclei
  - Cytosol (cytoplasm)
- Extracellular matrix

https://www.thieme.de/de/q.htm?p=opn/tp/308390101/978-1-62623-414-7_c001_v002&t=video

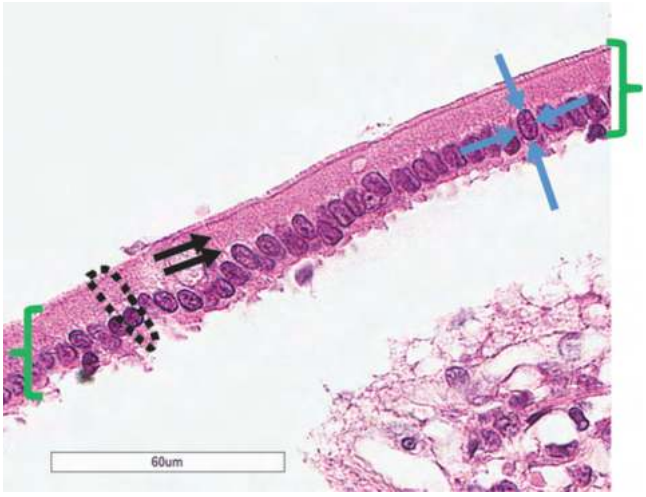

Fig. 1.5 **Intestine, indicating 30 to 50 epithelial cells (green brackets), a single cell (outlined), a border between two cells (black arrows), and nucleus (blue arrows).**

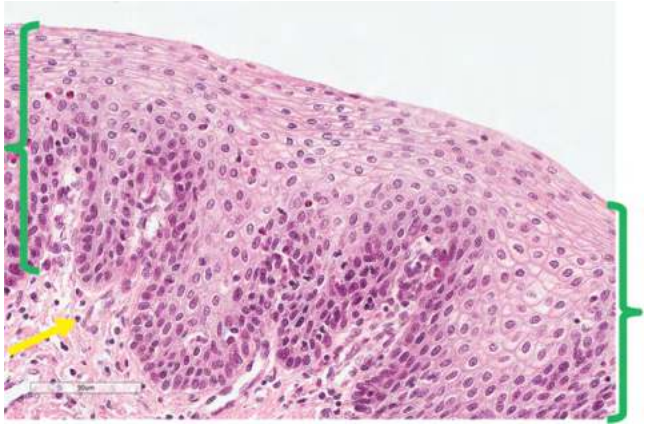

Fig. 1.6 **Pharynx, indicating epithelium (green brackets) and connective tissue cell (yellow arrow).**

Fig. 1.6 shows another image for practice. The region between the green brackets consists of over a hundred cells; most of them are cuboidal (square) or round. In most cases, the border between most cells is clearly visible, especially in the middle and upper regions. In places where the cell borders are not evident, they should be mentally drawn in.

Helpful Hint

The region in the bottom left in **Fig. 1.6**, below the tissue indicated by the green brackets, is slightly different. Here, the cells are smaller, with smaller nuclei; the nucleus of one cell is indicated by the yellow arrow. This type of tissue has more extracellular matrix than the tissue bounded by the green brackets. Therefore, the pink between the nuclei in this region includes both cytoplasm and extracellular matrix; the border between these is not visible. We'll look at why the tissues, cells, and nuclei are different in this region in subsequent chapters.

1.3 Slide Preparation

A complete understanding of the details of slide preparation is not necessary to study histology. However, understanding the procedure in general will make it easier to interpret histological slides.

The four major steps of slide preparation are:

1. Fixation
2. Embedding
3. Sectioning
4. Staining

1.3.1 Tissue Fixation

Tissue fixation terminates cell metabolism and prevents tissue degeneration. This can be accomplished with reagents that cross-link adjacent proteins (chemical fixation). **Fig. 1.7** shows a schematic representation of a heart in fixative solution.

Chemical fixation is time consuming and destroys protein structure. In some cases, if time or loss of enzymatic activity is a concern, tissues can be preserved by quick-freezing, a procedure in which the tissue is immersed in liquid nitrogen instead of a chemical fixative.

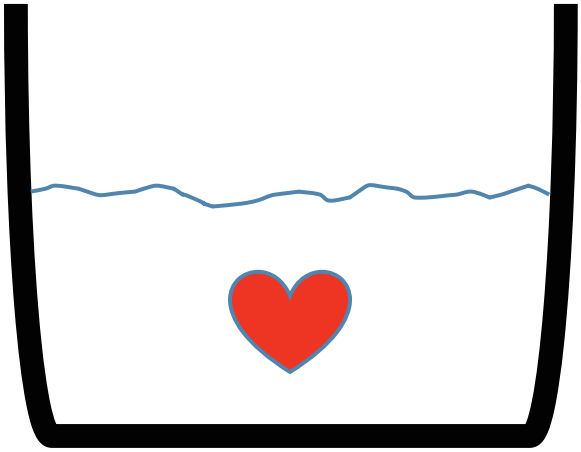

Fig. 1.7 **Sketch of tissue specimen in fixative.**

Clinical Correlate

Surgeons can biopsy tissues and send them to the pathology lab for quick analysis. Quick-freezing enables the pathologist to return the results of that analysis rapidly, even while the patient is still on the operating table. Surgical options can then be selected based on the results.

1.3.2 Embedding

Since organs and other specimens come in all shapes and sizes, fixed tissues are embedded in a medium that allows easy handling and sectioning. The result is a specimen within a block of embedding medium, which is solid (**Fig. 1.8**).

Helpful Hint

Embedding is not simply coating the outside of the tissue (**Fig. 1.9a**), but actual perfusion of the embedding substance into the tissue so that the tissue becomes part of the embedded block (**Fig. 1.9b**) and is less likely to fall apart on sectioning.

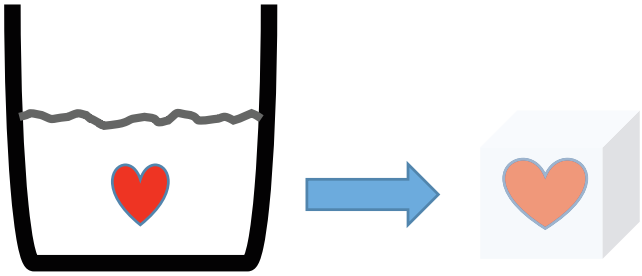

Fig. 1.8 **Tissue specimen in embedding media.**

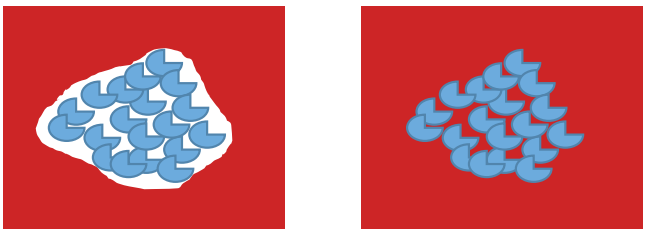

Fig. 1.9 **Schematic showing penetration of embedding media into tissue. (a) Medium does not embed into tissue. (b) Embedding media penetrating into the tissue.**

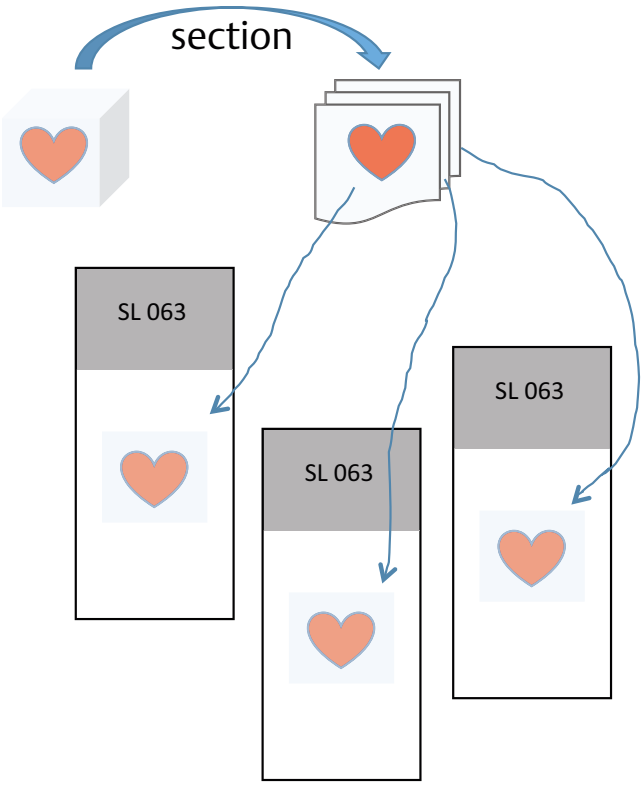

Fig. 1.10 **Tissue sectioning.**

1.3.3 Sectioning

The embedded tissue is then sectioned into thin (5–15 µm thick) slices using a microtome; the slices are mounted on glass slides (**Fig. 1.10**). **Fig. 1.10** shows only three sections from the sample. Hundreds of sections can be obtained from a single specimen. This process is called serial sectioning, since all the slides are generated from the same tissue.

1.3.4 Staining

Stains are used to enhance the visualization of cellular components. There are a number of procedures that can help visualize components on a slide; each will highlight a different cellular structure. This can range from relatively nonselective staining based on charge to very specific staining based on antibody-antigen interaction.

Fig. 1.11 shows an intestinal slide that either is unstained or did not stain very well. This image provides an idea of what an unstained piece of tissue looks like.

In the following paragraphs, two very common staining techniques are presented:

— Hematoxylin and eosin (H and E)
— Periodic acid–Schiff (PAS)

Hematoxylin and Eosin

Hematoxylin is a blue dye that localizes to negatively charged cell structures (e.g., deoxyribonucleic acid [DNA], ribonucleic acid [RNA]). Structures that bind to hematoxylin are referred to as **basophilic**.

Eosin is a red/pink dye that localizes to positively charged cell structures (e.g. proteins, mitochondria). Structures that bind to eosin are commonly referred to as **eosinophilic** or, sometimes, **acidophilic**.

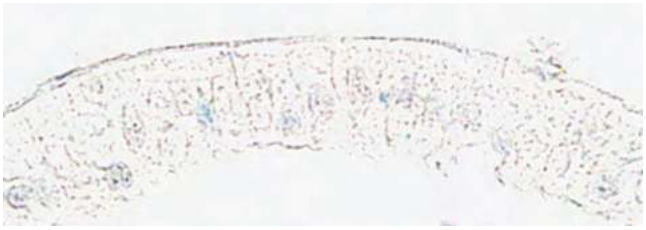

Fig. 1.11 **Unstained tissue.**

The liver cells in **Fig. 1.12** were stained with H and E. Note that hematoxylin staining of the negatively charged DNA molecules gives the nucleus a blue color (arrows), while the red/pink eosin stains proteins in the cytoplasm. Proteins in the extracellular regions (outlined) also are eosinophilic.

Ribosomes are cellular structures in the cytoplasm that synthesize proteins. Because ribosomes contain RNA, which is negatively charged and, therefore, basophilic, cells actively synthesizing large amounts of protein will demonstrate some hematoxylin stain in portions of the cytoplasm. This adds blue to the typically pink cytoplasm, creating a darker or purple shade, a feature referred to as **cytoplasmic basophilia**.

Fig. 1.13 is an image taken from the pancreas stained with H and E. In the pancreas and other organs, clusters of several cells are organized into structures called acini (black outline, **Fig. 1.13**). Most of the cells in an acinus are pyramid-shaped (yellow outline). In this image, most of the cells demonstrate cytoplasmic basophilia, especially surrounding the nucleus. Two cells with intense cytoplasmic basophilia are indicated by the yellow arrows.

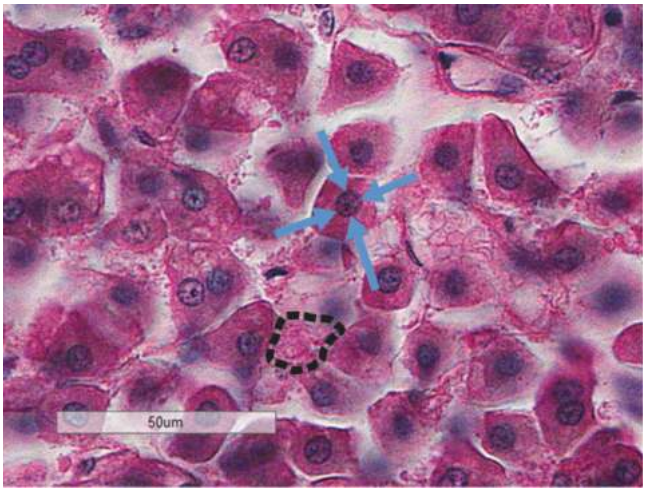

Fig. 1.12 **Liver tissue stained with H and E showing liver cell nucleus (blue arrows) and interstitial tissue (outlined).**

Video 1.3 H and E staining

Be able to identify:
— Hematoxylin and eosin (H and E) staining
  • Basophilic structures
  • Eosinophilic structures

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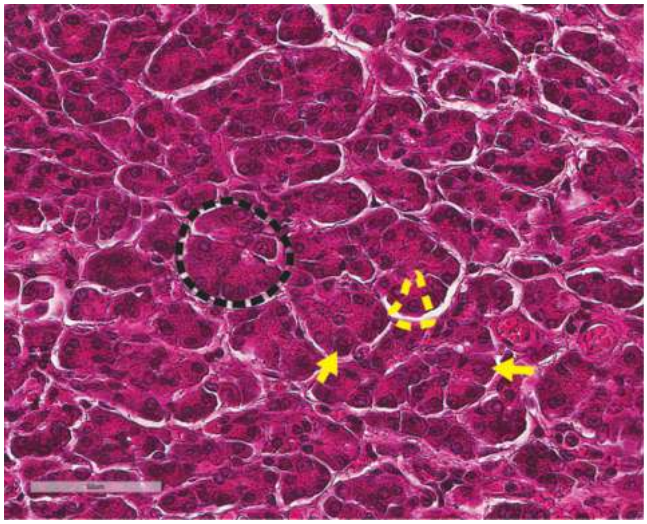

Fig. 1.13 **Pancreas stained with H and E, showing acinus (black outline), individual cell (yellow outline), and cytoplasmic basophilia (yellow arrows).**

Video 1.4 H and E staining with cytoplasmic basophilia

Be able to identify:
- H and E staining
  - Basophilic structures
    - Cytoplasmic basophilia
  - Eosinophilic structures

https://www.thieme.de/de/q.htm?p=opn/tp/308390101/978-1-62623-414-7_c001_v004&t=video

Fig. 1.14 shows another example of cells with cytoplasmic basophilia (blue arrows). Compare to cells with eosinophilic cytoplasm (green arrows).

Helpful Hint

While looking at **Fig. 1.14**, it's useful to point out the blood vessel located between the dotted lines. At the top and bottom, the vessel seems to disappear because it turns and leaves the plane of this section. Note the highly eosinophilic red blood cells, which lack nuclei (black arrow).

Cells with abundant cytoplasmic proteins show more intense eosinophilia. **Fig. 1.15a** shows cells with abundant mitochondria (black outline), while **Fig. 1.15b** is from skeletal muscle.

Just as the staining properties of the cytoplasm can provide clues to the function of a cell, the staining properties of the nucleus can also suggests a cell's relative activity. The genetic material in the nucleus, DNA, can be in a condensed (inactive) form called **heterochromatin** or in a decondensed (active) form called **euchromatin**. Condensed DNA in a cell will result in a smaller, more densely stained nucleus (black arrows, **Fig. 1.16**), whereas an active cell will have a larger, paler nucleus (yellow arrows).

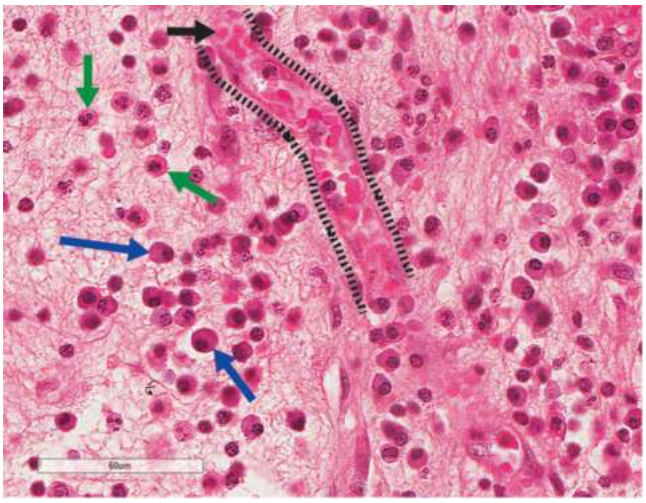

Fig. 1.14 **Inflamed tissue stained with H and E, showing cells with (blue arrows) and without (green arrows) cytoplasmic basophilia; this view also includes a blood vessel (dotted lines) with red blood cells (black arrow).**

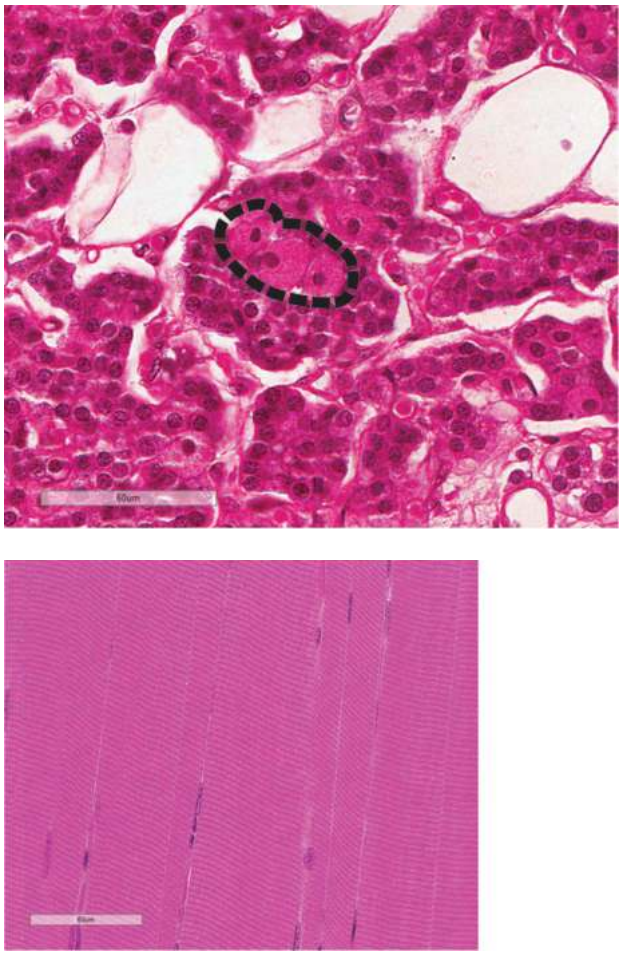

Fig. 1.15 **(a) Parathyroid gland stained with H and E, showing cells with intense cytoplasmic eosinophilia (outlined). (b) Skeletal muscle stained with H and E.**

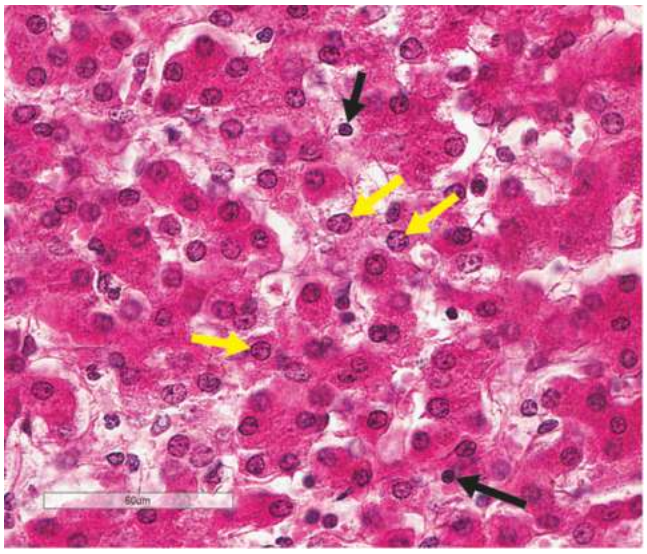

Fig. 1.16 **Liver, showing nuclei of active (yellow arrows) and inactive (black arrows) cells.**

Video 1.5: H and E nucleus

Be able to identify:
— A cell's activity based on its nuclear profile
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Periodic Acid–Schiff (PAS)

Periodic acid–Schiff (PAS) is a staining technique that highlights carbohydrates. Structures that react with this procedure are referred to as **PAS positive (PAS+)** and show up as a bright magenta (purple). The tissue is typically counterstained with a basic dye to highlight the nucleus. PAS+ structures within the cell are the Golgi apparatus, plasma membrane, and the mucus-containing vesicles of goblet cells. These structures will be discussed in detail in subsequent chapters.

Fig. 1.17 is a tissue stained with PAS. The lipid bilayer of plasma membranes includes a carbohydrate component called the glycocalyx; therefore, cell borders are more clearly defined in tissue stained with PAS. The cells that stain intensely positive with PAS (black arrows) contain abundant secretory vesicles in the cytoplasm, which are filled with glycoproteins. In addition, other structures in the cells and the extracellular matrix are PAS+. More details about all these PAS+ structures will be discussed in upcoming chapters.

Video 1.6: PAS

Be able to identify:
— A tissue stained with PAS
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1.3.5 Artifacts

A final topic regarding preparation of slides is **artifacts**. Artifacts are features of a prepared slide that are not present in the original sample but are introduced during tissue preparation.

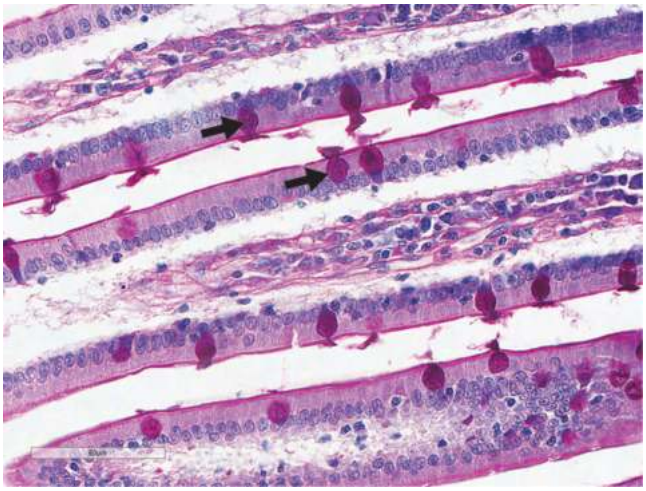

Fig. 1.17 **Jejunum stained with PAS. Cells rich in carbohydrate, called goblet cells, are indicated (black arrows).**

The absence of staining in the cytoplasm suggests the presence of specific molecules in the tissue. Some components of the cell do not take up a particular stain very well. For example, liver cells (hepatocytes) contain glycogen, which does not stain well in routine H and E tissue preparation. In **Fig. 1.18**, varying levels of glycogen in the hepatocytes results in slightly more or less staining in the cytoplasm. Although all the cells show some degree of lack of cytoplasmic staining, cells with more abundant clear areas, and thus more glycogen, are outlined.

Some cellular components also wash away from the sample during tissue fixation. This leaves a clear area that is a clue to what once existed in the original tissue. In the adrenal cortex, lipid droplets are stored in the cytoplasm of cells as precursors to the steroid hormones the cells produce. These lipid droplets wash away on routine tissue preparation. In **Fig. 1.19**, the degree of cytoplasmic staining varies in different regions; the cells with the most washed-out cytoplasm are in the center.

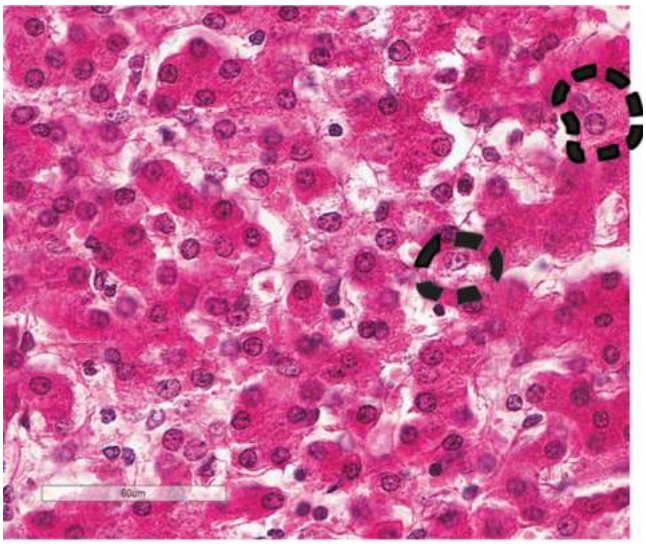

Fig. 1.18 **Liver, showing cells with pale-staining regions (outlined) due to abundant glycogen.**

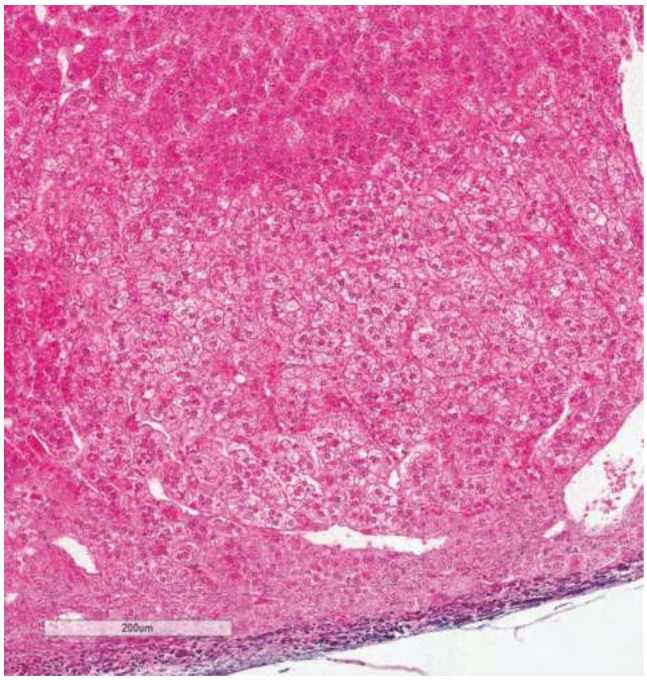

Fig. 1.19 **Adrenal gland, showing pale-staining region (center) due to abundant lipid droplets.**

Video 1.7 H and E liver

Video 1.8 H and E adrenal gland

Be able to identify:
— A cell's cytoplasmic contents based on appearance (for now, at least list possibilities of what could be there)
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https://www.thieme.de/de/q.htm?p=opn/tp/308390101/978-1-62623-414-7_c001_v008&t=video

Helpful Hint

The preceding text states that glycogen is pale because it does not take up H and E stain well, while the video states that glycogen washes away during routine tissue preparation. Both explanations have been given to explain the pale appearance of glycogen-containing areas in liver cells, as well a similar appearance of carbohydrates in other cells (e.g., mucus, described in a later chapter). It's likely that the former explanation is correct, since similarly prepared slides stained with PAS demonstrate these carbohydrate components of the cell. For practical purposes, it makes no difference; understanding the significance of pale regions in cells stained with H and E (i.e., what they represent) is sufficient.

Another artifact of sample preparation is tissue shrinkage. This occurs in most routine preparations of tissue samples, though some fixation procedures minimize tissue shrinkage. Not all tissues in a sample shrink to the same extent. When one tissue shrinks more than an adjacent tissue, the tissues pull away from each other, creating spaces within the sample. For example, in the image of cartilage in **Fig. 1.20**, the extracellular

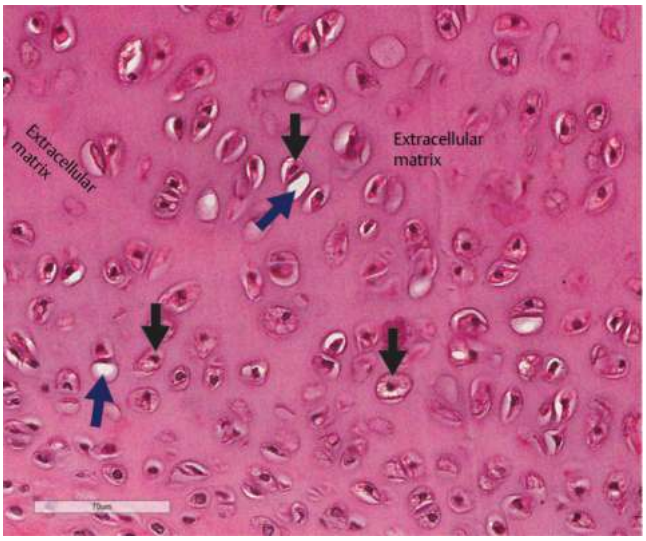


Fig. 1.20 **Hyaline cartilage, showing cells (black arrows) and empty lacuna (blue arrows), and extracellular matrix.**

matrix is semisolid, so it shrinks less than the cells (black arrows) that are embedded within it. This creates holes (called lacunae), in which the shrunken cells are located. In many places (blue arrows), the cells have fallen out of their lacuna during sectioning.

Video 1.9 Lacunae

Be able to identify:
— Cells in lacunae
https://www.thieme.de/de/q.htm?p=opn/tp/308390101/978-1-62623-414-7_c001_v009&t=video

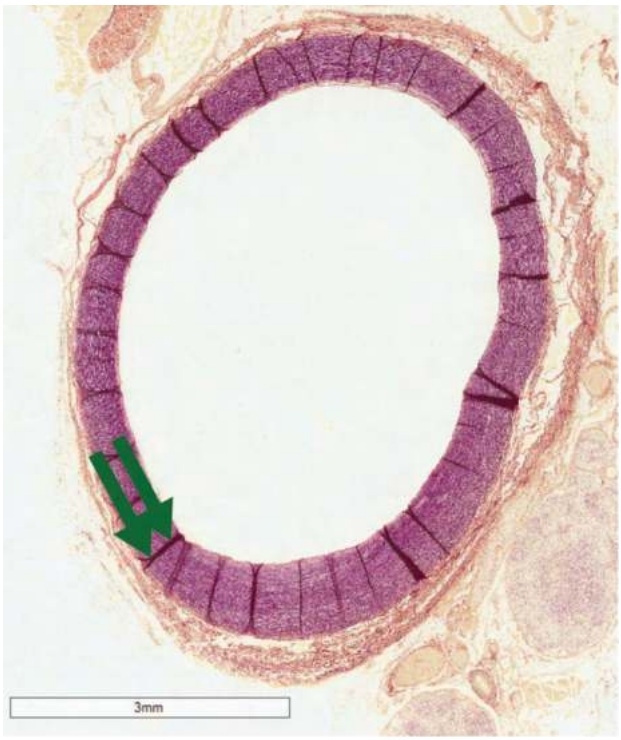
Helpful Hint

Although artifacts are representations on slides that aren't really present in real tissues, in some cases, features created during tissue preparation can actually be helpful in tissue identification. In this example, since only cartilage and bone have shrunken cells within lacunae, recognizing this feature helps in identifying these tissues.

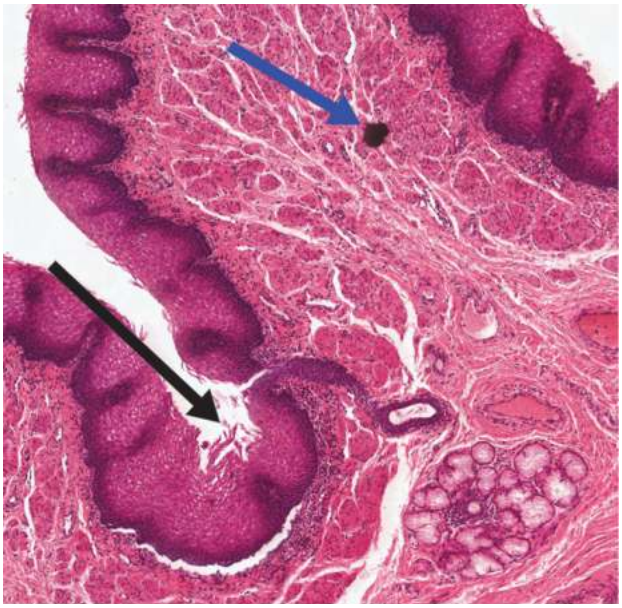
Fig. 1.21 shows two examples of artifacts that are not so helpful. **Fig. 1.21a** shows a cross section of an elastic artery. During tissue shrinkage, the vessel folded up on itself, creating dark bands (green arrows) that are not real structures but are simply areas of overlapped tissue. **Fig. 1.21b** features torn tissue (black arrow) and a piece of debris (blue arrow).

1.4 Chapter Review

Histology is the study of biologic structures too small to be visible with the naked eye. To study them, microscopes are used to magnify the image of a structure. Alternatively, tissues can be digitally scanned and presented to the user electronically (digital slides). To prepare a tissue for visualization, it must be fixed, embedded, sectioned, and stained. A wide



a



b

Fig. 1.21 **(a) Aorta, showing folds in the tissue (green arrows). (b) Esophagus, showing tear (black arrow) and debris (blue arrow).**

variety of stains can be used to highlight specific structures. The most commonly used histologic stain is hematoxylin and eosin (H and E). In H and E–stained slides, nuclei are blue and the cytoplasm and extracellular matrix are typically pink. However, cells synthesizing large amounts of proteins have abundant ribosomes in the cytoplasm and will demonstrate cytoplasmic basophilia on H and E staining. Another common histological stain is periodic acid–Schiff (PAS), which stains carbohydrates. Artifacts are features introduced during tissue preparation and include clear spaces due to lack of staining, washing out of tissue components, or tissue shrinkage. These artifacts may be useful in determining cellular function or in identifying tissue types.

Questions and Answers

An online-only section of questions and answers accompanying this chapter is hosted on Thieme's MedOne Education site: https://medone-education.thieme.com. Use the code on the media page at the front of this book to gain access. An institutional license to the site is required to access these questions in an interactive format, and individual users are required to register for an individual account to track results.

2 The Nucleus

After completing this chapter, you should be able to:

- Identify, at the light microscopic level, each of the following
  - Cell
    - Nucleus
    - Nuclear envelope (nuclear membrane)
    - Nucleolus
    - Euchromatin
    - Heterochromatin
  - Phases of the cell cycle
    - Interphase
    - Mitosis
      - Prophase
      - Metaphase
      - Anaphase
      - Telophase
- Identify, at the electron microscopic level, each of the following
  - Cell
    - Nucleus
    - Nuclear envelope (nuclear membrane)
    - Nuclear pores
    - Nucleolus
    - Euchromatin
    - Heterochromatin
- Evaluate the status (activity) of a cell based on the appearance of the nucleus
- Predict the effect of agents that interfere with the cell cycle
- Evaluate the activity of a tissue based on the presence of mitotic figures

2.1 Typical Nucleus

The previous chapter introduced the main features of the cell visible using light microscopy. In **Fig. 2.1**, an entire cell is outlined in green, and a **nucleus** from another cell is shown (blue arrows). In H and E staining, nuclei are basophilic, while the eosinophilic **cytoplasm** includes everything in the cell except the nucleus.

That chapter focused on large nuclei for convenience. This chapter takes a more detailed look at the nucleus.

First off, note that nuclei come in different shapes and sizes (**Fig. 2.1**, black and blue arrows). In most cases, nuclear shape reflects the shape of the cell; for example, a round cell will have a round nucleus (blue arrows), while a spindle-shaped cell (yellow outline) has a longer, narrower nucleus.

Second, and maybe more importantly, the size of the nucleus typically reflects the activity level of the cell. Active cells are often involved in either DNA or RNA synthesis, which represent cell division or protein expression, respectively. Therefore, the nucleus provides a clue as to whether the cell is very active (blue arrows) or relatively inactive (black arrows).

To examine the **nucleus** in more detail, it helps to select a large nucleus (black arrows, **Fig. 2.2**). The outer border of the nucleus is the **nuclear envelope** (tips of the black arrows), which consists of two lipid bilayers that cannot be distinguished at the light microscopic level. The content of the nucleus is referred to as the **nucleoplasm**. In this cell, most of the nucleoplasm is pale; this color represents **euchromatin**, which is decondensed DNA involved in either DNA replication or RNA transcription. The large dark structure in the center of the nucleus is the **nucleolus**, which is the site of ribosome synthesis. The other smaller dark regions, and in particular the dark structures just under the nuclear envelope, represent **heterochromatin**, inactive condensed DNA.

Helpful Hint

Just as nuclear size provides a sense of the activity of the cell, the relative amount of euchromatin in the nucleus will indicate how active a cell is, because it represents DNA that is in the decondensed (active) state. Smaller nuclei (yellow arrow) are composed mostly of heterochromatin, suggesting a less active cell.

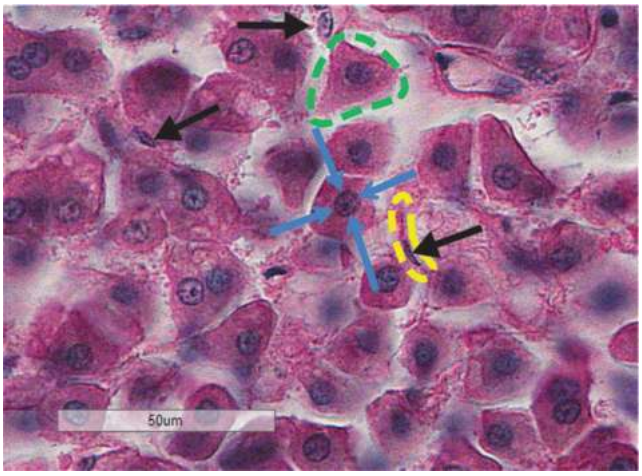


Fig. 2.1 **Liver, showing liver cell (green outline), an active nucleus (blue arrows), inactive nuclei (black arrows), and an inactive cell (yellow outline).**

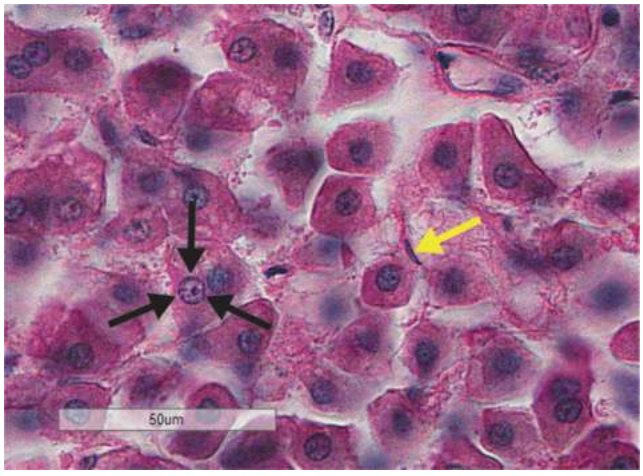


Fig. 2.2 **Liver, showing active nucleus (black arrows) and inactive nucleus (yellow arrow).**

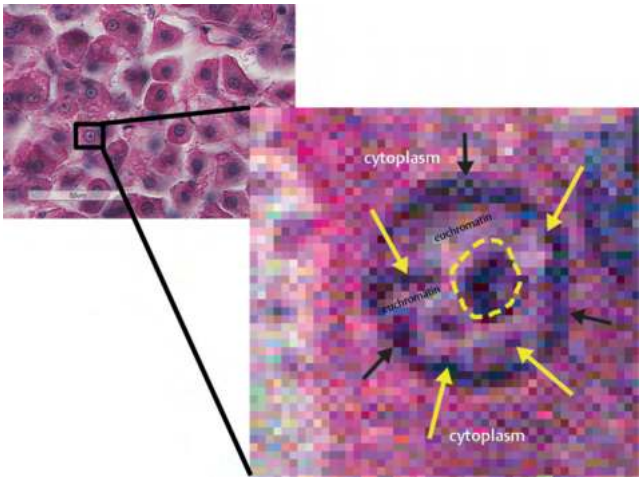


Fig. 2.3 **Liver cell nucleus, showing cytoplasm, euchromatin, heterochromatin (yellow arrows), nucleolus (yellow outline), and nuclear envelope (black arrows).**

Fig. 2.3 shows a region from **Fig. 2.2** artificially enlarged. This enlarged image is pixelated but allows for more accurate labels to indicate the parts of the nucleus:

- Nuclear envelope: not visible; location is the tips of the black arrows
- Nucleolus: outlined in yellow
- Euchromatin: pale areas indicated by text
- Heterochromatin: yellow arrows

Video 2.1 Typical nucleus

Be able to identify:
— Nucleus
— Nuclear envelope
— Nucleolus
— Euchromatin
— Heterochromatin
https://www.thieme.de/de/q.htm?p=opn/tp/308390101/978-1-62623-414-7_c002_v001&t=video

Helpful Hint

Note that even though **Fig. 2.3** is enlarged and pixelated, it can be used as a reference to go back to **Fig. 2.2** or any other image of the nucleus and see its details.

2.2 Electron Microscopy of the Nucleus

As mentioned in **Chapter 1**, electron microscopy is an imaging technique similar to light microscopy, but it uses electrons instead of light to create the image, which provides greater magnification and resolution of the specimen.

In the electron micrograph (EM) shown in **Fig. 2.4**, the approximate border of a single cell is outlined in red, and its nucleus is outlined in yellow. Note that numerous structures within the cytoplasm that are not evident in light microscopy are readily evident in electron micrographs; these will be discussed in detail in the next chapter.

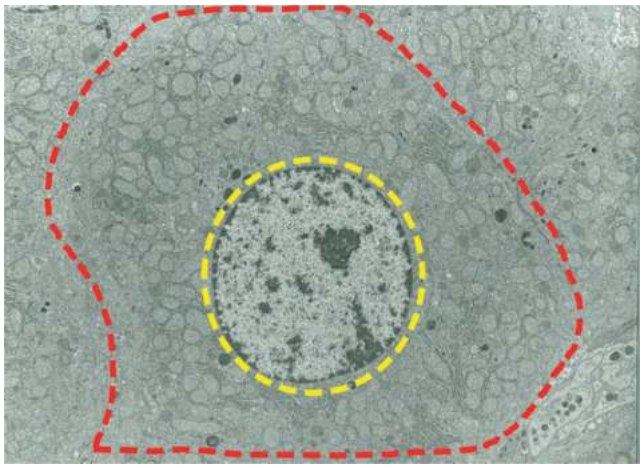


Fig. 2.4 **Liver, electron micrograph, showing entire cell (red outline) and nucleus (yellow outline). Image courtesy of Robert and Emma Lou Cardell.**

Fig. 2.5 shows an enlarged view of the nucleus from the cell in **Fig. 2.4**, revealing greater detail of structures that were seen in the light micrograph:

- Nuclear envelope: barely visible, location is the tips of the black arrows
- Nucleolus: outlined in yellow
- Euchromatin: pale areas indicated by text
- Heterochromatin: yellow arrows

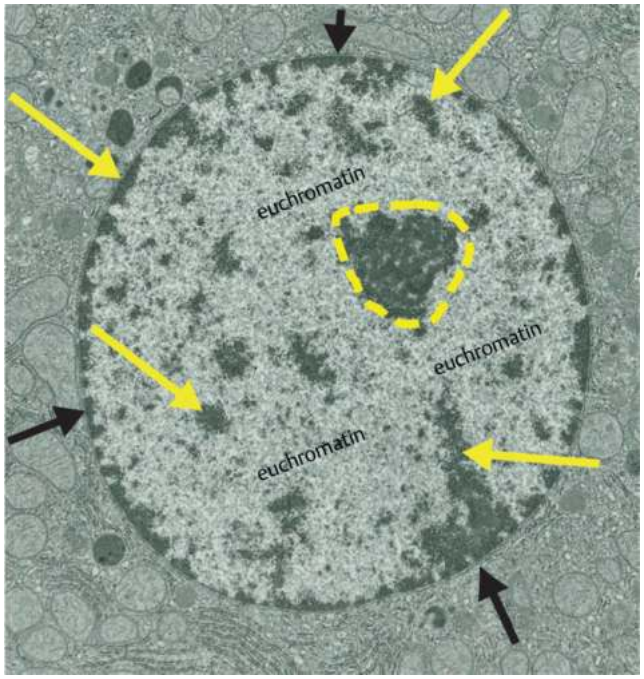


Fig. 2.5 **Liver, electron micrograph, showing euchromatin, nuclear envelope (black arrows), heterochromatin (yellow arrows), nucleolus (yellow outline). Image courtesy of Robert and Emma Lou Cardell.**

Helpful Hint

Note that in this EM the nuclear envelope can be seen a little more clearly (but not quite). Heterochromatin is scattered throughout this nucleus, but mostly against the inner part of the nuclear envelope. Abundant euchromatin suggests that the cell is either an active cell (expressing numerous genes) or undergoing DNA synthesis to prepare for cell division. This is a liver cell, so it is indeed very active metabolically. The nucleolus is prominent, suggesting assembly of ribosomes necessary for protein synthesis.

Fig. 2.6 shows a more detailed view of the edge of the nucleus. The **nuclear envelope** consists of two membranes (two lipid bilayers); each shows up as a linear structure at this magnification. The outer membrane is indicated by the series of red arrows and is studded with round structures (ribosomes). The inner membrane (yellow arrows) is less distinct due to the heterochromatin (labeled and at 3) adjacent to it. The space between the membranes is referred to as the perinuclear cisterna (1). The black arrows indicate **nuclear pores**, openings in the nuclear envelope that allow molecular trafficking between the nucleus and cytoplasm. The label 2 indicates rough endoplasmic reticulum in the cytoplasm, discussed in the next chapter.

2.3 The Cell Cycle

Many cells in a specimen are in the process of cell division. While part of this involves the physical division of one cell into two, a cell must spend significant time preparing for this division, including replication of organelles and, most importantly, the genetic material (DNA). Therefore, actively dividing cells are typically in one of four phases of the **cell cycle** (see **Fig. 2.7**):

- **G_1** (growth phase): A cell that has just divided grows under the influence of resource availability and hormonal signals. During the G_1 phase, the cell assesses its environment and will divide if conditions are optimal. If the conditions are right, the cell will enter the S phase, at which time it will be committed to progress through the remainder of the cell cycle back to the G_1 phase.
- **S** (synthesis phase): This is the phase in which the genetic material is duplicated.
- **G_2** (growth phase): Here the cell assesses the duplicated genetic material and prepares for cell division.
- **M** (mitosis): This is the visible manifestation of cell division, in which the single cell divides into two. **Mitosis** specifically consists of the division of the nucleus and itself has phases, highlighted in the next section. Mitosis is capped by **cytokinesis**, which is the division of the cytoplasm.

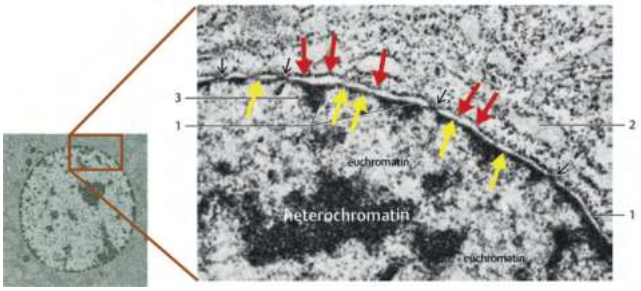


Fig. 2.6 **Nuclear envelope, showing inner (yellow arrows) and outer (red arrows) nuclear membranes, nuclear pores (black arrows), euchromatin, heterochromatin (3), perinuclear cisterna (1), and rough endoplasmic reticulum (2).**

Note that the first three phases of the cell cycle, G_1, S, and G_2, are collectively referred to as **interphase**; cells in these phases are indistinguishable by light microscopy.

Many of the cells in tissues such as the epidermis of the skin or the gut epithelium are continually regenerating to replace damaged cells and, therefore, are continuously progressing through the cell cycle. However, cells in many tissues stop dividing and may differentiate into specialized cell types. These cells leave the G_1 phase and enter a phase referred to as **G_0**. For example, after development is complete, neurons in the brain stop dividing and function to transmit action potentials; these cells are considered to be in the G_0 phase because they are no longer actively dividing. In other tissues, cells may enter the G_0 phase temporarily, returning to the cell cycle when necessary. For example, fibroblasts in the skin are relatively quiescent but can be stimulated to divide in order to regenerate damaged tissue.

2.3.1 Mitosis

Fig. 2.8a–h depict a cell proceeding through mitosis. **Fig. 2.8a** is a cell in interphase; recall that cells in G_1, G_0, S, and G_2 are all in interphase and will all appear similar in routine light microscopy. The oval structure is the nucleus, and the nuclear envelope is at the tips of the blue arrows. Note the nuclear material in interphase in this cell is largely euchromatin, so individual chromosomes are not seen. At least one or two of the visible structures in the nucleus are nucleoli. The cytoplasm is very poorly stained, but it is easiest to see in **Fig. 2.8h**.

The phases of mitosis are:

1. **Prophase** (**Fig. 2.8b**): The nuclear envelope breaks apart, and the chromatin condenses, forming visible chromosomes. Structures called centrioles that assemble the mitotic spindle that guides the chromosomes during mitosis move to opposite poles of the cell (in this image, top and bottom), but these are not visible in this image.

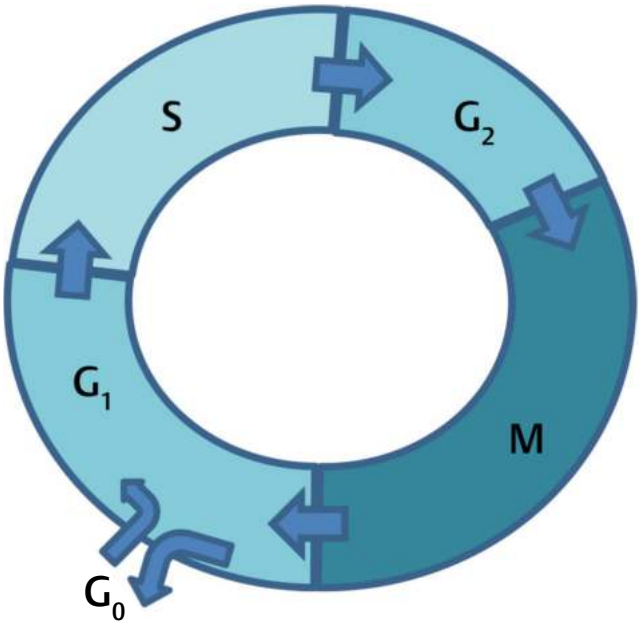


Fig. 2.7 **The cell cycle.**

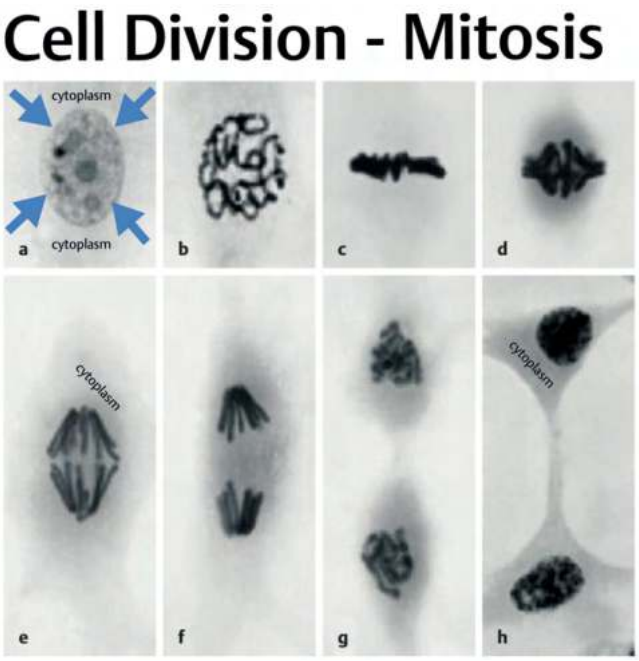


Fig. 2.8 **Mitosis. (a) Interphase. (b) Prophase. (c) Metaphase. (d–f) Anaphase. (g–h) Telophase. The blue arrows in (a) indicate the nuclear envelope, which breaks down in prophase.**

2. **Metaphase** (**Fig. 2.8c**): The chromosomes line up along the middle of the cell, called the equator.
3. **Anaphase** (**Fig. 2.8d–f**): The chromosomes are moved to opposite poles of the cell.
4. **Telophase** (**Fig. 2.8g–h**): The chromosomes have reached their respective poles; division of the cytoplasm (cytokinesis) occurs, the nuclear envelopes reform, and the chromatin decondenses.

Helpful Hint

Mitosis is a continuum, so many cells seen in tissue specimens are somewhere between the four phases outlined above.

To study the phases of mitosis, it is useful to select a specimen containing many rapidly dividing cells, such as embryonic tissue; the following figures show sections from whitefish embryos. Recall that, even in rapidly dividing tissues, many cells will be in interphase, not mitosis. In **Fig. 2.9**, most of the cells are in interphase. One cell in this image is outlined; the nucleus is the large, basophilic structure in the center of the cell. Note the distinct nuclear envelope; the chromatin is a mixture of euchromatin and heterochromatin (no visible chromosomes).

Helpful Hint

It might be tempting to think that many of the cells in this image that do not show nuclei are in some phase of mitosis. However, this is not the case because, as the next set of images will demonstrate, mitotic cells have distinct chromosomes.

The reason those cells lack a visible nucleus in this image is that the plane of section passed through a portion of the cell that did not include the nucleus.

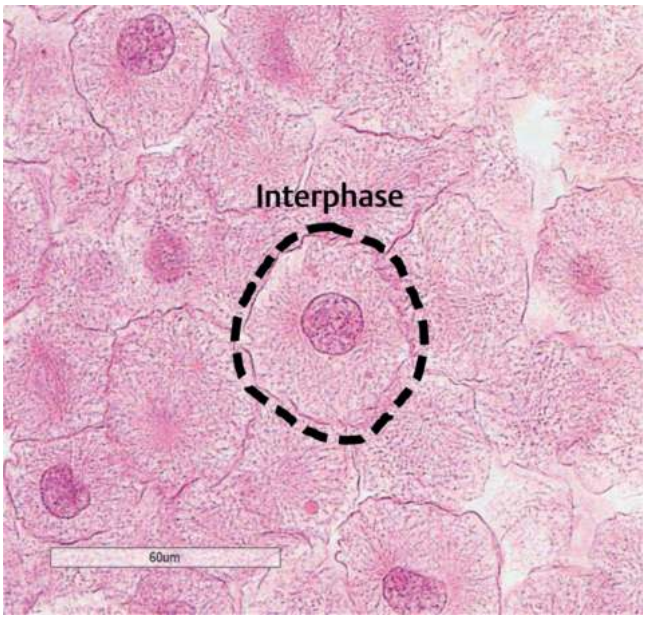


Fig. 2.9 **Cells from whitefish embryo in interphase.**

The first phase of mitosis is prophase (**Fig. 2.10a**). Here, the nuclear envelope breaks down, and the chromatin condenses to form visible chromosomes (purple speckles in the center of the cell).

Metaphase is highlighted by the alignment of the chromosomes along the equator (**Fig. 2.10b**). The cage-shaped structure is the **mitotic spindle** (black arrows), which is made up of **microtubules** and is responsible for movement of the chromosomes.

Anaphase, shown in **Fig. 2.11a**, is highlighted by movement of the chromosomes to opposite ends of the cell (poles).

Once the chromosomes reach the poles, telophase begins (**Fig. 2.11b,c**). During this phase, the cytoplasm of the cell divides (cytokinesis). Eventually, the nuclear envelope will reappear, and the chromatin will decondense. The resulting cells will enter interphase (G_1).

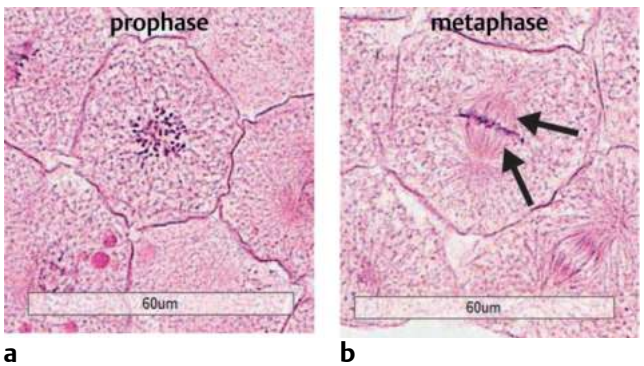
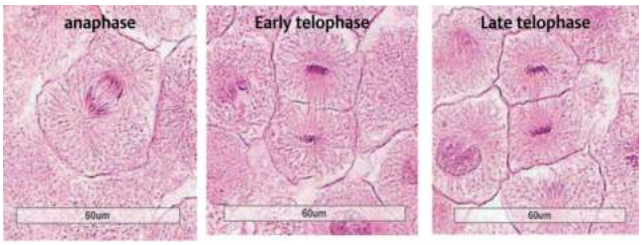


Fig. 2.10 **Cells from whitefish embryo in (a) prophase and (b) metaphase.**



a b c

Fig. 2.11 **Cells from whitefish embryo in (a) anaphase and (b,c) telophase.**

Video 2.2 Phases of mitosis

Be able to identify:
— Interphase
— Prophase
— Metaphase
— Anaphase
— Telophase
https://www.thieme.de/de/q.htm?p=opn/tp/308390101/978-1-62623-414-7_c002_v002&t=video

2.4 Mitotic Figures in Tissues

In select tissues such as the whitefish embryo, it's easy to find cells in mitosis and to identify the specific phase of mitosis. However, in most other tissues, cells in mitosis are not as common, even in proliferative tissues such as the epidermis of the skin.

In **Fig. 2.12**, taken from the pharynx, most of the cells are in interphase (three cells in interphase are outlined in black). Note the nucleus and the cytoplasm of these cells.

Outlined in yellow is a cell in mitosis, characterized because it lacks a nuclear envelope and has visible dense chromosomes. It is likely that this cell is in late telophase or has just finished cytokinesis and the chromosomes of the progeny cells have yet to decondense. Because it is often difficult to determine a specific stage of mitosis for cells in tissues, they are often referred to as **mitotic figures**.

Clinical Correlate

In many histologic and pathologic specimens, recognizing mitotic figures is useful in determining tissue type or type of pathology.

Video 2.3 Mitotic figures

Be able to identify:
— Mitotic figures
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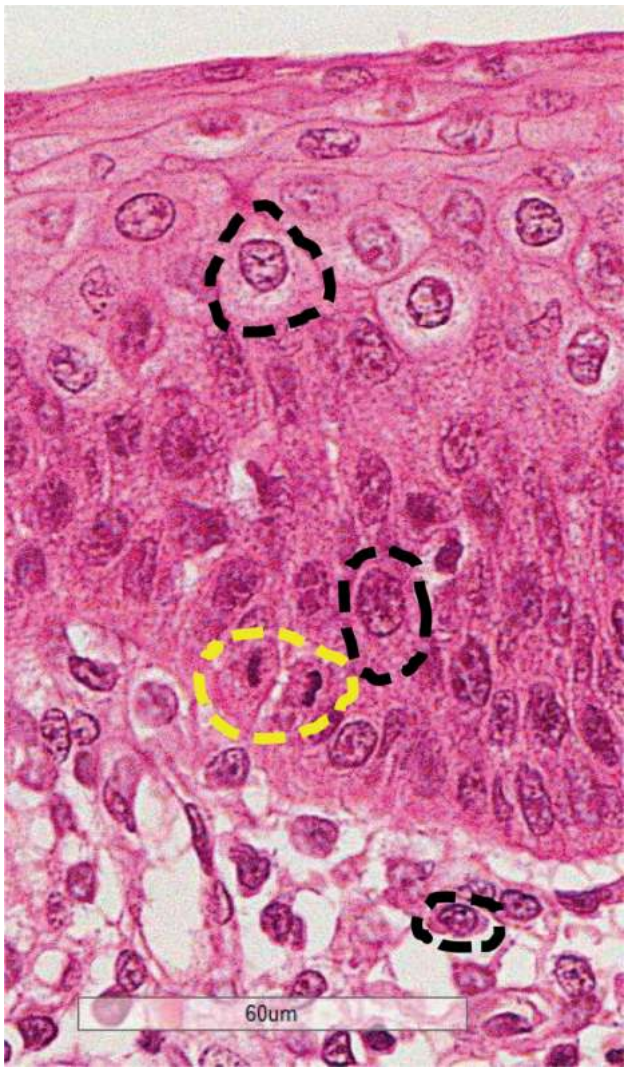

Fig. 2.12 **Pharynx, showing mitotic figure (yellow outline) and cells in interphase (black outlines).**

2.5 Chapter Review

The nucleus is typically the largest and most visible organelle in the cell. It houses the genetic material, DNA, in the form of chromosomes. Portions of these chromosomes are either in a decondensed or condensed form, seen in light and electron microscopy as euchromatin or heterochromatin, respectively. Nucleoli within the nucleus are the sites of ribosome assembly. Actively dividing cells progress through a cell cycle that is highlighted by phases of growth (G_1, G_2), DNA synthesis (S), and cell division (mitosis, M). The phases of mitosis are prophase, metaphase, anaphase, and telophase. Mitotic figures can be seen in tissue sections and underscore the mitotic activity of a normal or pathologic tissue.

Questions and Answers

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3 The Cytoplasm

After completing this chapter, you should be able to:

- Identify, at the light microscopic level, each of the following:
  - Cell
    - Plasma membrane
    - Cytoplasm
  - Cytoplasmic basophilia
- Identify, at the electron microscopic level, each of the following:
  - Cell
    - Plasma membrane
    - Cytoplasm
    - Mitochondria
    - Rough endoplasmic reticulum
    - Smooth endoplasmic reticulum
    - Golgi apparatus
    - Lysosomes
- Outline the function of the cellular structures listed
- Predict the organelles that would be prominent in a cell or tissue based on its function
- Predict the appearance of cells in light micrographs based on their appearance in electron micrographs, and vice versa

3.1 Cytoplasm

Fig. 3.1 shows a typical cell (liver cell). The arrows have been moved to indicate the **cytoplasm**, which is the main focus of this chapter. Recall that, due to high protein content, the cytoplasm in most cells is eosinophilic when stained with H and E. Cells that are actively synthesizing proteins, on the other hand, have cytoplasmic basophilia. In fact, certain regions in these liver cells have purple hues in the cytoplasm, reflecting active protein synthesis.

3.2 The Plasma Membrane

Recall that the outer structure of a cell is the **plasma membrane (cell membrane)**, which forms the boundary between the cell and its extracellular environment. The plasma membrane is composed of a lipid bilayer with associated proteins and a carbohydrate component on the external surface called the **glycocalyx**. In light micrographs, the plasma membrane is seen as a linear structure. In **Fig. 3.1**, the edge of the cell indicates the location of the plasma membrane, even though no obvious line is visible. In **Fig. 3.2**, the plasma membranes are seen as visible lines; these lines are, in fact, two plasma membranes (one from each cell) adjacent to each other. In many cases, such as the bottom left portion of **Fig. 3.2**, the plasma membrane is not clearly visible, so the cytoplasm of those cells cannot be distinguished from the extracellular matrix.

Video 3.1 Plasma membrane

Be able to identify:

- Cell (plasma) membrane
- Cytoplasm
- Extracellular matrix

https://www.thieme.de/de/q.htm?p=opn/tp/308390101/978-1-62623-414-7_c003_v001&t=video

Fig. 3.3 shows an electron micrograph of a single cell from the lower left region of the image in **Fig. 3.2**. In the light micrograph, only the nucleus of the cell within the box is visible, and the cytoplasm of that cell and surrounding extracellular matrix are indistinguishable. In the electron micrograph, the plasma membrane is visible as a thin line that surrounds the cell (blue arrows). In some regions the plasma membrane is distinct, while in other regions it becomes blurred or obscured by intra- or extracellular structures (e.g., bottom right of the cell). In electron micrographs, numerous structures, both within and outside the cell, are easily seen.

3.3 Cytoplasmic Organelles

The basic structure of the cell was introduced in **Chapter 1**. The remainder of this chapter will focus on the major organelles found within the cytoplasm (mitochondria, ribosomes, rough

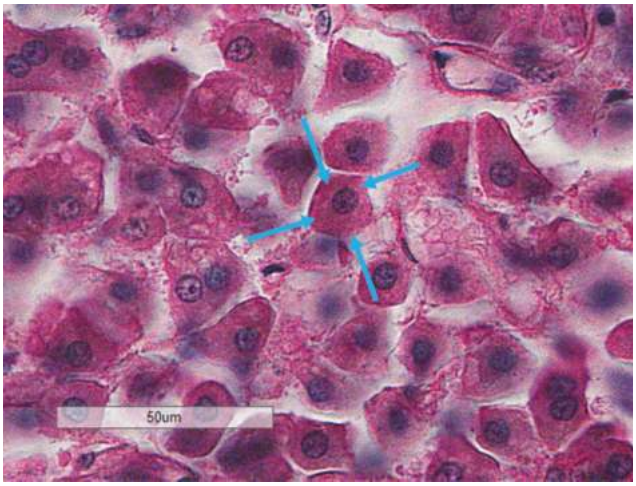

Fig. 3.1 **Typical cell from the liver. Blue arrows indicate cytoplasm.**

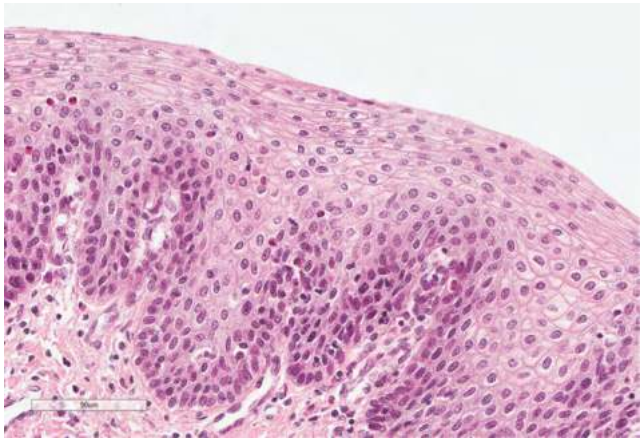

Fig. 3.2 **Typical cells from the stratified squamous epithelium of the pharynx. Note the plasma membranes visible in the upper layers.**

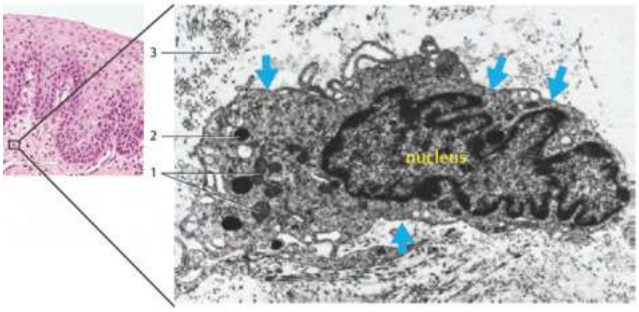

Fig. 3.3 **Typical cell (electron micrograph), showing cell membrane (arrows) and nucleus (labeled). Mitochondria (1), secretory granules (2), collagen fibers (3).**

and smooth endoplasmic reticulum, Golgi apparatus, lysosomes). Other organelles are mentioned here and discussed in more detail as they are encountered in subsequent chapters.

Many organelles in the cytoplasm are bounded by lipid bilayers with a composition similar to the plasma membrane. These membranes are shown in **Fig. 3.4** as thin lines and, like the plasma membrane, are visible on electron micrographs.

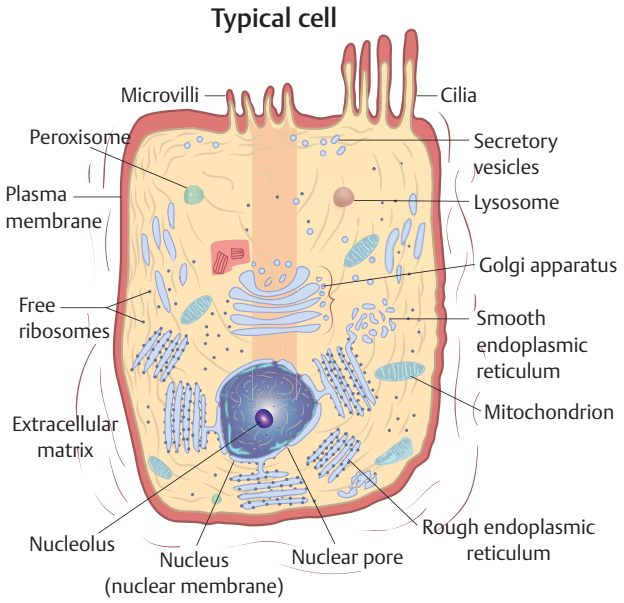

Fig. 3.4 **Illustration of a typical cell.**

3.3.1 Nucleus

Before discussion of organelles in the cytoplasm, recall that the **nuclear envelope** is composed of two lipid bilayers (indicated by red and yellow arrows, respectively, in **Fig. 3.5**). Most cellular organelles are bounded by one lipid bilayer, the exceptions being the nucleus and mitochondria, which are bounded by two bilayers.

3.3.2 Mitochondria

Mitochondria are the powerhouses of the cell, responsible for oxidizing pyruvate and fatty acids to generate high energy bonds in the form of adenosine triphosphate (ATP).

Fig. 3.6 is a drawing of the membrane structure of a mitochondrion. Like the nucleus, mitochondria are bounded by two membranes (two lipid bilayers). These membranes are termed the **outer membrane**, which is the outer boundary of the mitochondrion, and the **inner membrane**, which is typically thrown up into folds (**cristae**) to increase surface area.

Outside the mitochondrion is the cytoplasm. The substance within the inner membrane is the **matrix**, while the **intermembrane space** is the thin region between the inner and outer membranes.

In electron micrographs, mitochondria are readily recognized as oval structures (**Fig. 3.7**), though some are more elongated, while others are seen in cross section and appear round. At this magnification it is challenging to differentiate the inner and outer membranes distinctly when they are adjacent to each other.

Fig. 3.8 shows a drawing of a mitochondrion (**Fig. 3.8a**) and a corresponding electron micrograph showing multiple mitochondria (**Fig. 3.8b**). The folds (cristae) of the inner membrane (blue arrows) appear as double lines because the inner membrane folds back on itself. In contrast, double lines at the outer edge of the mitochondria (red arrows) are adjacent inner and outer membranes.

The cristae of the inner membrane of many mitochondria are flattened, so they appear linear in electron micrographs. However, in some cells, the inner membrane has tubular cristae, as shown in **Fig. 3.9**. These mitochondria are typical of steroid-secreting cells and will be helpful in identifying such cells in later chapters.

When looking at electron micrographs, it is always useful to take a moment and consider the size of cellular structures to maintain perspective. In **Fig. 3.10** a single cell is outlined in red, and the nucleus is outlined in yellow. This cell has over 50 mitochondria visible here; a single mitochondrion is outlined in purple. Compare the size of a mitochondrion to the size of the nucleus, and to the size of the entire cell.

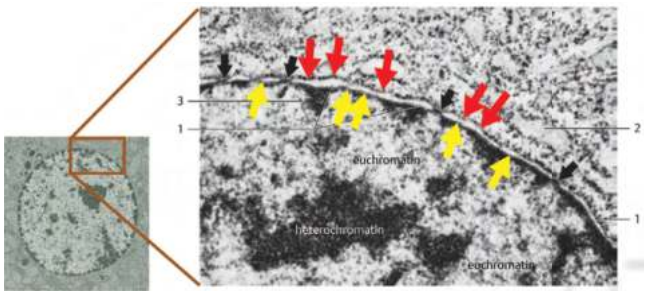

Fig. 3.5 **(Left) Electron micrograph showing complete nucleus. Image courtesy of Robert and Emma Lou Cardell. (Right) More highly magnified electron micrograph of part of the nucleus corresponding to the inset region of the micrograph at left. Red and yellow arrows: outer and inner bilayers of nuclear envelope, respectively. Black arrows: nuclear pores. Perinuclear cisterna (1), rough ER (2), heterochromatin (3).**

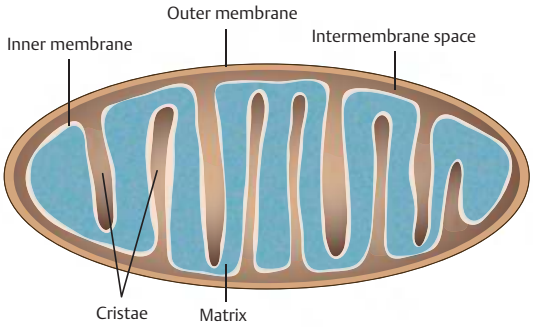

Fig. 3.6 **Illustration of mitochondrion.**

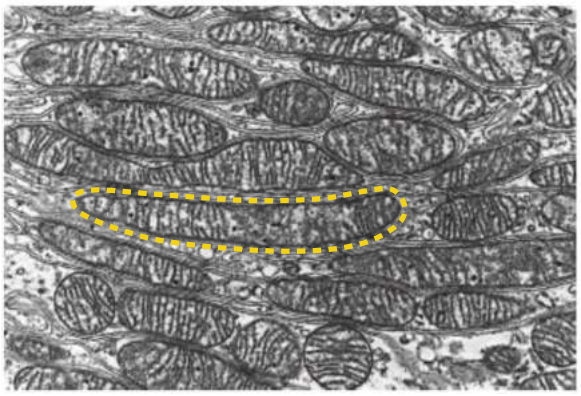

Fig. 3.7 **Electron micrograph of a cell with multiple mitochondria; one is outlined in yellow.**

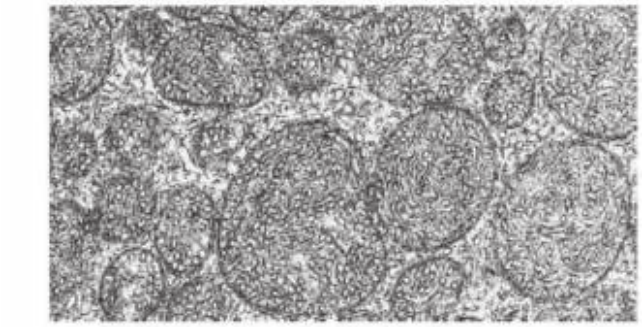

Fig. 3.9 **Electron micrograph of mitochondria from a steroid-secreting cell showing tubular cristae.**

3.3.3 Ribosomes

Ribosomes are the site of protein synthesis. In electron micrographs (**Fig. 3.11**), ribosomes appear as dense, round structures about 30 nanometers in diameter (indicated by 2 and 3 in the figure). Compare the size of ribosomes to the mitochondrion (1 in the figure). The ribosome at 3 in **Fig. 3.11** is a **free ribosome**,

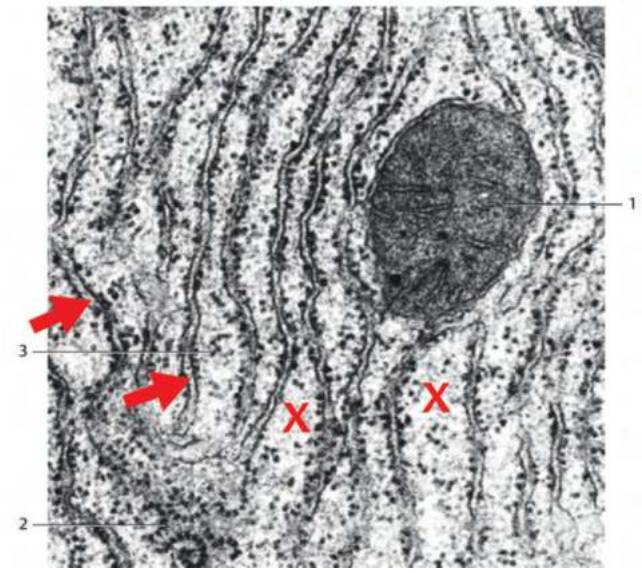

Fig. 3.11 **Electron micrograph showing a mitochondrion (1), ribosomes attached to rough endoplasmic reticulum (RER) (2), free ribosomes (3). Arrows mark the lumen of RER, and cytoplasm is marked with Xs.**

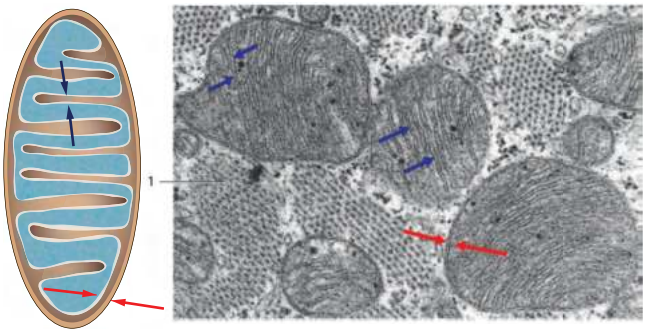

Fig. 3.8 **(a) Drawing and (b) electron micrograph of mitochondria showing double lipid bilayers at cristae (blue arrows) and inner and outer membranes (red arrows). Myofilament bundles (1).**

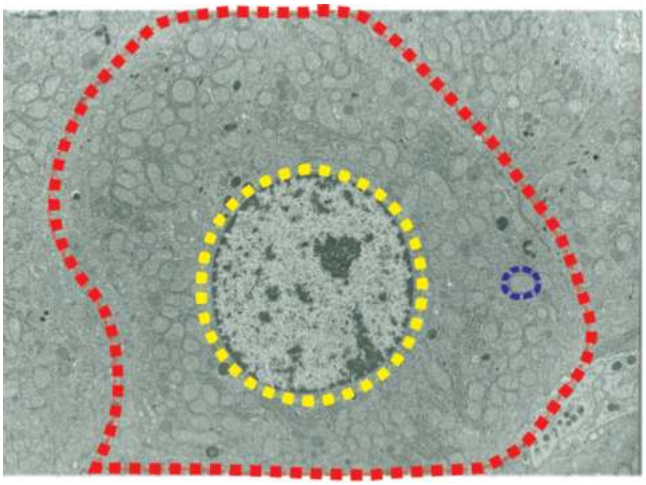

Fig. 3.10 **Electron micrograph of liver cell, showing cell (red outline), nucleus (yellow outline), and one mitochondrion (purple outline). Image courtesy of Robert and Emma Lou Cardell.**

which synthesizes proteins destined to reside in the cytoplasm (marked with a red X in the figure), nucleus, mitochondria, or peroxisomes. The ribosomes at 2 in the figure are attached to the **rough endoplasmic reticulum** (RER; see **Fig. 3.4**), which will be discussed in the next section.

Helpful Hint

Because they cover a wide range of sizes, nuclei, mitochondria, and ribosomes are helpful to determine the relative size of other structures in electron micrographs.

3.3.4 Rough Endoplasmic Reticulum

Proteins synthesized by ribosomes found on rough endoplasmic reticulum are either inserted into the membrane of the rough ER, or placed in the RER lumen during translation. From there, vesicles containing these proteins bud from the rough ER and fuse with the Golgi apparatus, which processes these proteins (e.g., adding carbohydrate). This post-translational modification is crucial for proper function of these proteins. After processing through the Golgi, these proteins are moved to their destination in the cell via vesicles that bud from the Golgi apparatus. The rough ER/Golgi synthesizes and processes proteins that are destined for the following locations: lysosomes, secretory vesicles, the plasma membrane, the extracellular space, and resident proteins of the rough ER and Golgi.

Observing RER in Electron Microscopy

In **Fig. 3.11**, the RER is best seen immediately to the left of the mitochondrion (1), where the membranes of the RER are more distinct and clearly studded with ribosomes. The lumen of the RER is marked for orientation purposes by the tips of red arrows in the figure. Proteins translated by ribosomes on RER are inserted into the lumen or RER membrane as they are synthesized.

Helpful Hint

Note that ribosomes stud the outside of the RER membrane, which should provide orientation of this organelle relative to the cytoplasm.

In **Fig. 3.12**, the RER is the major organelle in the outlined region. At this lower magnification, it is more challenging to see individual ribosomes clearly. However, note that RER typically forms stacks of membranes, making identification of RER possible even at lower magnifications in which ribosomes are not visible.

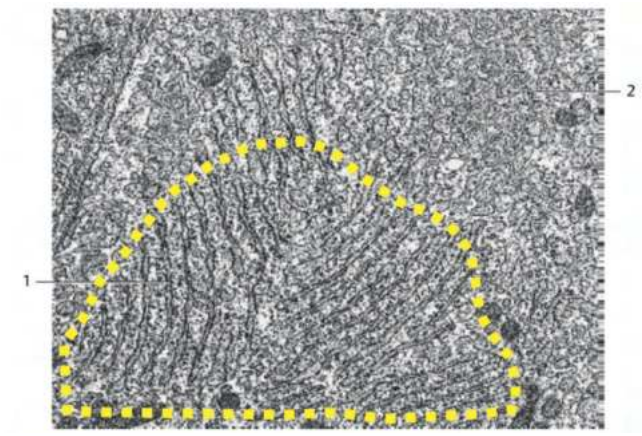

Fig. 3.12 **Rough endoplasmic reticulum outlined (and at 1) on electron micrograph. Smooth endoplasmic reticulum (2) also shown for comparison.**

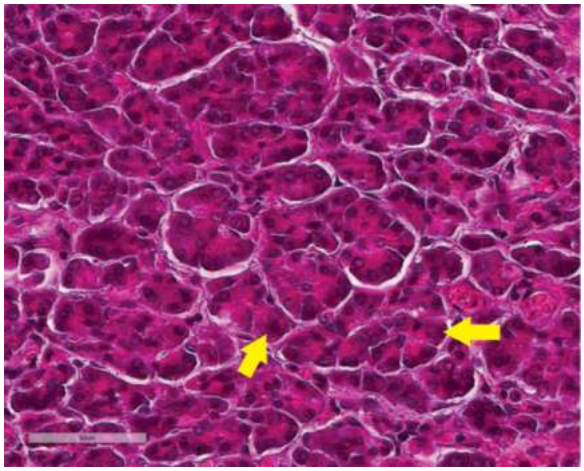

Fig. 3.14 **Exocrine pancreas, showing cytoplasmic basophilia (arrows).**

Helpful Hint

Histologists and pathologists often use food references to describe histological features of tissues and cellular structures. With respect to RER, a stack of pancakes may serve this purpose, maybe with chocolate chips on top and in between the pancakes.

RER is typically, but not always, seen as stacks of membranes in electron micrographs. In the cell shown in **Fig. 3.13,** the lumen of the RER (at 1) is dilated with proteins. Close examination shows ribosomes studding the membranes of the RER. This dilation of the RER is characteristic of cells that are secreting large amounts of proteins.

Observing RER in Light Microscopy

Individual ribosomes and membranes of RER are not visible using light microscopy. However, clues to the amount of RER in the cell can be suggested by the staining properties of the cell. Cells actively synthesizing proteins contain numerous ribosomes. This is particularly true of cells secreting large amounts of proteins, which will have abundant rough endoplasmic reticulum, causing **cytoplasmic basophilia**, discussed in **Chapter 1** (e.g., the

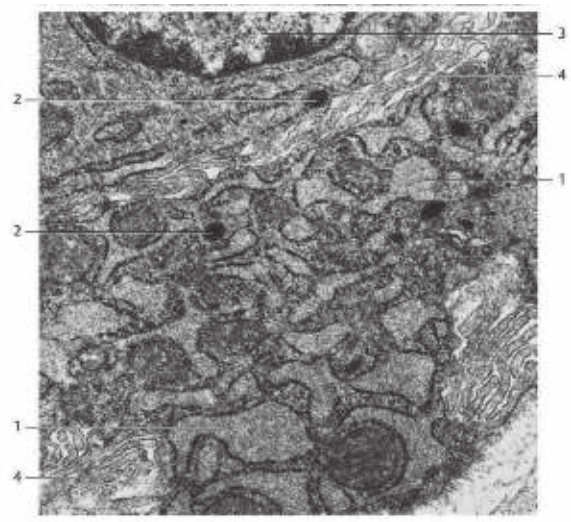

Fig. 3.13 **Rough endoplasmic reticulum in a cell actively secreting protein. Lumen of RER (1), secretory granules (2), nucleus (3), intercellular space (4).**

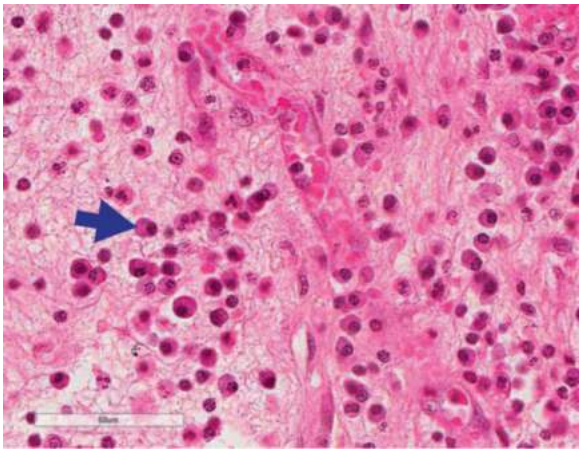

Fig. 3.15 **Plasma cell (arrow) in inflammation, showing cytoplasmic basophilia.**

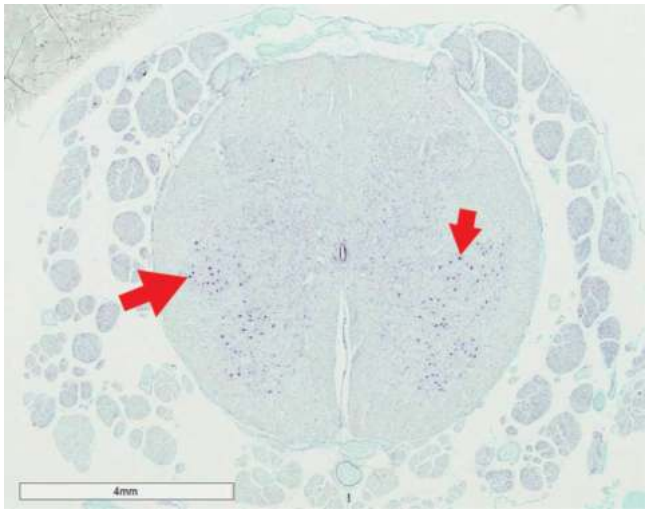

Fig. 3.16 **Cross section of spinal cord under Nissl stain. Motor neurons are indicated by the arrows.**

pancreas cells secreting digestive enzymes in **Fig. 3.14** and the plasma cells secreting antibodies in **Fig. 3.15**).

Special stains other than H and E can help visualize the presence of RER. **Fig. 3.16** is a section of the spinal cord. For this discussion, it is not important to know the details of the spinal cord (**Chapter 15**); suffice it to say that motor neurons in the spinal cord are quite large. Even in this low-magnification image these motor neurons can be seen as small purple dots (red arrows). **Fig. 3.17** shows an expanded view of a region containing these motor neurons; one neuron is outlined in blue. The large, oval, pale region in the center of the cell is the nucleus, while the round, dark structure in the middle of the nucleus is the nucleolus (tip of the red arrow). This is a special stain (**Nissl stain**), which stains RNA, a major component of ribosomes. That is why it strongly stains the nucleolus (where ribosomes are made; **Chapter 2**) and RER. If the cytoplasm of a cell stains intensely with Nissl stain, it is because that cell has abundant RER for protein synthesis. The other strongly stained round structures in **Fig. 3.17** are nuclei of glial cells, which support the neuron. The pale, tubular structure just to the right of and below the motor neuron is a blood vessel.

Video 3.2 Nissl stain of motor neurons

Be able to identify:
- Nissl substance (RER)
- Nucleus (review)
- Nucleolus (review)

https://www.thieme.de/de/q.htm?p=opn/tp/308390101/978-1-62623-414-7_c003_v002&t=video

3.3.5 Golgi Apparatus

Proteins synthesized by the RER undergo some additional processing (e.g., adding carbohydrate molecules). This **post-translational modification** is crucial for proper function of these proteins. This processing is performed by the **Golgi apparatus**, a complex of several stacked disk-shaped compartments called cisternae, enclosed by membranes similar to the ER. The typically three to eight cisternae of the Golgi complex work as a single unit. Vesicles containing newly synthesized proteins bud off from the RER and

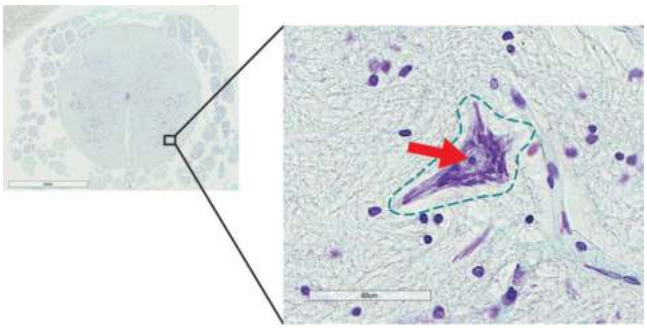

Fig. 3.17 **(Left) Cross section of spinal cord under low magnification; (right) motor neuron at high magnification under Nissl stain, showing neuron (outlined) with RER-rich cytoplasm and active nucleolus (arrow).**

fuse with the membranes of the first cisterna of the Golgi complex on one side, termed the **cis face**. Proteins progress through the Golgi apparatus via **transport vesicles** that bud off each cisterna and fuse with the next, undergoing chemical modifications along the way. Mature proteins in the last cisterna are finally released from the **trans face** of the Golgi apparatus in vesicles.

In **Fig. 3.18**, the Golgi apparatus is outlined. As mentioned, it is similar to RER in that it consists of stacks of flattened compartments enclosed by membranes. However, it is not studded with ribosomes. The cisternae typically bow slightly, so that the cis face is the convex side of the Golgi apparatus (2), while the trans face is the concave side (3). Transport vesicles are numerous (1), and a portion of the nucleus of this cell is indicated (4).

Helpful Hint

Looking for the absence of ribosomes to identify the Golgi apparatus (relative to RER) is a logical thing to do. However, it helps to have other features to differentiate these organelles in low-magnification images. As mentioned, note that the Golgi tends to bow (bend) slightly and has numerous vesicles at its periphery and trans face (larger vesicles typically are at the trans face). In addition, the rims of the cisternae of the Golgi apparatus are slightly dilated relative to the middle regions. RER may happen to have some of these features, but it is less likely to have all of them. So, taken collectively, these features can assist in correctly identifying the Golgi apparatus.

When considering the appearance of the Golgi apparatus on light micrographs, it is useful to re-examine cells with cytoplasmic basophilia. Recall that cytoplasmic basophilia is a feature of cells

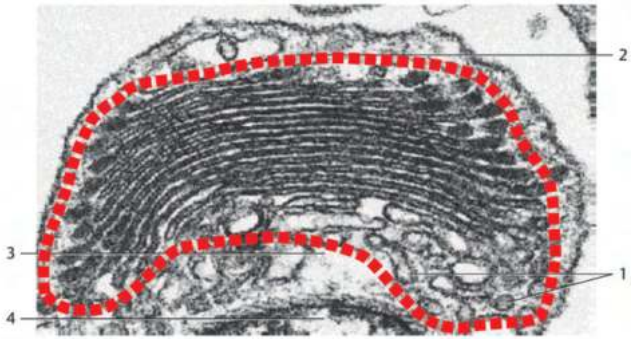

Fig. 3.18 **Electron micrograph showing prominent Golgi apparatus. Transport vesicle (1), cis-face (2), trans-face (3), nucleus (4).**

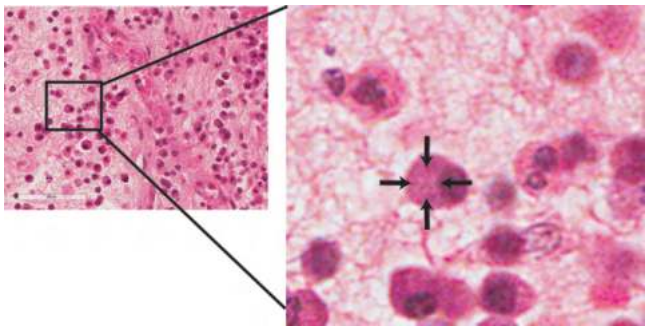

Fig. 3.19 **Plasma cell in inflammation. Golgi apparatus appears as a paler spot against the basophilic cytoplasm (Golgi ghost; outlined by the tips of the arrows).**

that have abundant RER. Since the RER and Golgi apparatus work together to synthesize and process proteins, it makes sense that a cell that has abundant RER would also have a prominent Golgi. This is indeed the case; however, the presence of the Golgi is not obvious in every cell, and even when visible, it is subtle at best.

Fig. 3.19 shows an artificially magnified region of an image of inflamed tissue. In the magnified region, the cell in the center demonstrates cytoplasmic basophilia because it is actively producing immune proteins. The nucleus of this cell is located in the right portion of the cell. In the center of the cell is the location of the Golgi apparatus (outlined by the tips of the arrows). Because the Golgi lacks ribosomes, this region demonstrates less cytoplasmic basophilia than the rest of the cytoplasm, and it appears slightly pale (relative to the rest of the cytoplasm). Many histologists refer to this phenomenon as the **Golgi ghost**.

Helpful Hint

Recognizing the Golgi ghost in light micrographs is subtle, more art than science and is typically not a crucial component of recognizing a protein-synthesizing cell, since the cytoplasmic basophilia is more reliable.

3.3.6 Smooth Endoplasmic Reticulum

Smooth endoplasmic reticulum (SER) is a network of membrane-bound organelles that are elongated, tube-shaped, and not studded with ribosomes. In **Fig. 3.20**, SER occupies most of the cell (only a portion of the cell is shown here). SER has several functions, mostly related to lipid biosynthesis (e.g., steroid production) or glycogen metabolism. In addition, it has a major role in detoxification.

Helpful Hint

Because the SER is tube-shaped (think of a tree or ginger root), sections through the SER will generate round or short tube-shaped sections, as opposed to the longer, flattened stacks of membrane typically seen with RER or Golgi. Also, the lack of ribosomes is helpful in identifying SER.

Smooth and rough ER are continuous. In fact, they can be converted one to another, largely by the addition or removal of ribosomes. This is common in cells such as hepatocytes of the liver, which play a major role in both protein synthesis and detoxification.

In **Fig. 3.21**, which shows the same field shown in **Fig. 3.12**, recall that the RER is indicated by 1. SER is in the outlined region (2), but it is also seen to the upper left of the RER. Note that the two reticula are continuous.

3.3.7 Lysosomes

Lysosomes are membrane-bound structures containing digestive enzymes, involved in intracellular breakdown of senile organelles or of exogenous cells (e.g., bacteria) and molecules ingested by the cell. Lysosomes newly formed by budding from the Golgi apparatus are often called primary lysosomes, which become secondary lysosomes when they fuse with structures to be degraded. Secondary lysosomes are larger, and because they contain breakdown products in different stages of degradation, they are relatively easy to identify in a cell because their lumen is heterogeneous. Lysosomes typically are about half the size of mitochondria, but can be larger.

In **Fig. 3.22**, two large secondary lysosomes are shown (one is outlined), and a smaller primary lysosome is marked by the black arrow at right.

3.3.8 Other Cellular Organelles

There are other organelles within the cell that are not necessary to identify on electron micrographs at this time. These include:

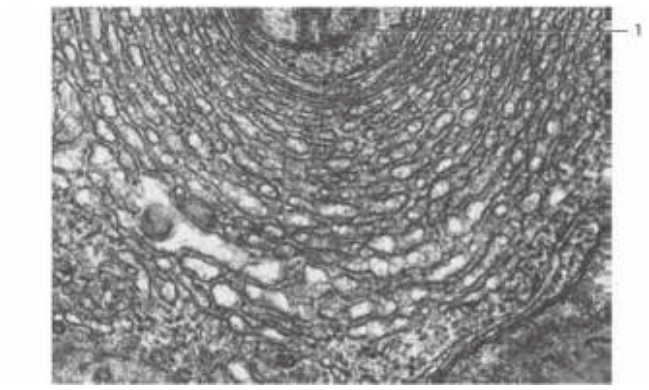

Fig. 3.20 **Electron micrograph showing smooth endoplasmic reticulum filling entire cell. Nucleus of the cell is indicated (1).**

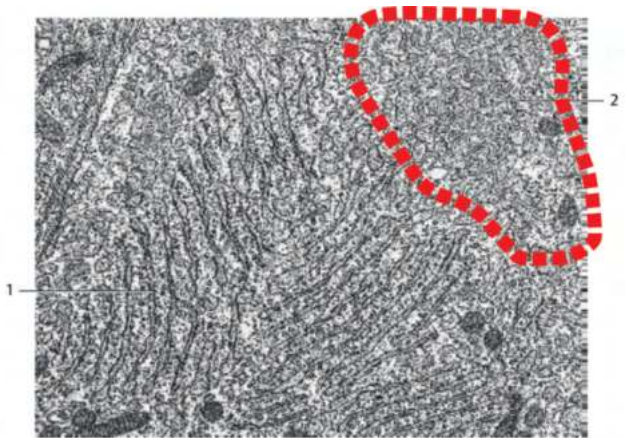

Fig. 3.21 **Electron micrograph showing smooth endoplasmic reticulum (outlined and at 2). Rough endoplasmic reticulum (1) also shown for comparison.**

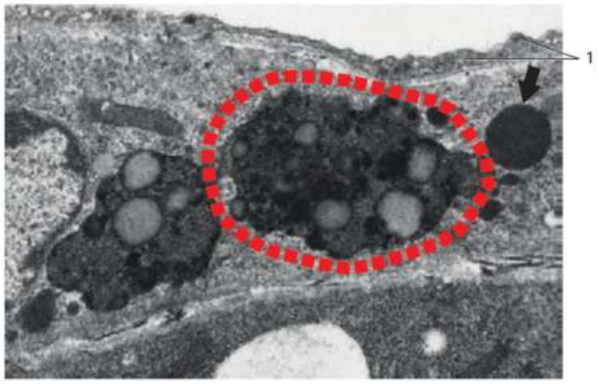

Fig. 3.22 **Electron micrograph showing lysosomes (outlined and black arrow). Capillary endothelium (1).**

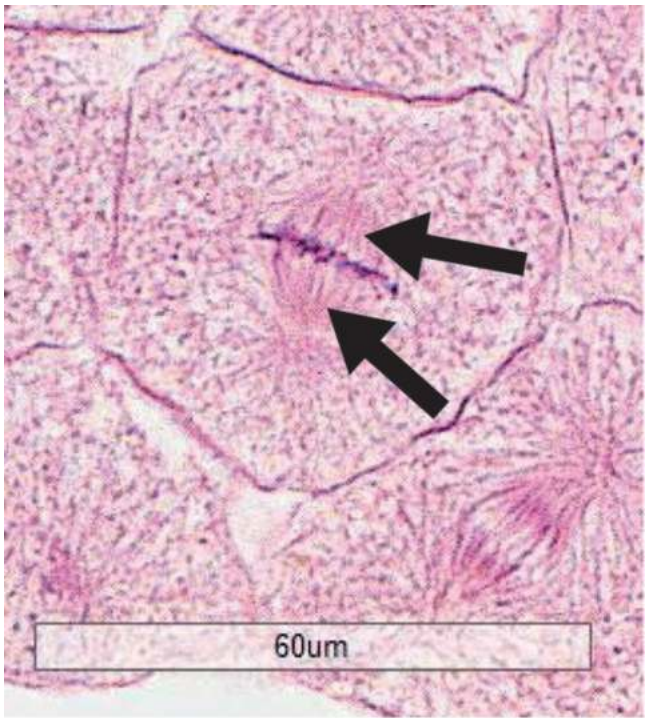

Fig. 3.23 **Mitotic spindle (black arrows).**

1. **Secretory vesicles** are products of the RER and Golgi apparatus; they contain proteins or other molecular products to be secreted into the extracellular matrix.
2. **Peroxisomes** have a major role in detoxification.
3. **Lipid droplets** are for storage of fatty substances, seen prominently in adipose tissue.
4. **Glycogen** is a form of stored carbohydrate; glycogen particles are similar in appearance to ribosomes, but cluster together, often associated with SER.
5. The **cytoskeleton** is a filamentous network within the cell that is responsible for cell structure, cell movement, and movement of cellular organelles or chromosomes. There are three components to the cytoskeleton: **actin microfilaments**, **intermediate filaments**, and **microtubules**. The mitotic spindle (involved in chromosome movement during mitosis) is composed of microtubules (**Fig. 3.23**, arrows), while cytokinesis (division of the cytoplasm) is a process driven by actin filaments.

We'll come back to some of these organelles as they arise in subsequent chapters.

Helpful Hint

Staining of the cytoskeleton generates some of the most impressive images in cell biology—do a Google image search.

3.4 Chapter Review

The outer boundary of the cell is a lipid bilayer called the plasma (cell) membrane. The part of the cell excluding the nucleus is the cytoplasm, which is composed of the cytosol and organelles. Many organelles are bounded by a lipid bilayer similar to the plasma membrane, including rough and smooth endoplasmic reticulum, the Golgi apparatus, lysosomes, peroxisomes, and secretory vesicles. Mitochondria and the nucleus are unique because they are bounded by two membranes. Other structures in the cell are not membrane bound, including free ribosomes, glycogen, lipid droplets, and components of the cytoskeleton. Each structure in the cell plays a role in the functions of the cell. For example, the rough ER and Golgi apparatus are involved in protein synthesis and processing.

Questions and Answers

An online-only section of questions and answers accompanying this chapter is hosted on Thieme's MedOne Education site: https://medone-education.thieme.com. Use the code on the media page at the front of this book to gain access. An institutional license to the site is required to access these questions in an interactive format, and individual users are required to register for an individual account to track results.