6 Histopathology

Objectives

In this chapter we will study

- some of the factors leading to cellular injury and necrosis;
- the effects of cell damage or cell death on tissues and organs; and
- how histotechnologists and histopathologists study diseased tissues.

Cellular Injury and Tissue Damage

Cells are vulnerable to injury by such chemical and physical factors as oxygen deprivation *(hypoxia),* free radicals, toxic chemicals, nutritional imbalances, temperature extremes, acids and bases, trauma, and radiation. Cells can often respond to such disturbances by making homeostatic adjustments in their metabolism, but severe disturbances may cause structural changes that can be observed microscopically. The study of such structural changes and the diagnosis of disease from histological evidence—that is, the clinical application of histology—is called **histopathology.**

Irreversible damage to cell structure and function leads to cell and tissue *necrosis*—pathological death, as compared to *apoptosis,* the "programmed death" of cells that were destined to die in the service of normal bodily development and function (see *A&P,* p. 198). Some of the causes of cellular injury are described here.

Hypoxia

Hypoxia, or oxygen deficiency, is the most common cause of cell death. It can result from **ischemia** (deficient blood flow to a tissue), respiratory disorders such as emphysema that interfere with oxygen pickup by the blood, or various kinds of poisoning that interfere with the blood's ability to transport oxygen (carbon monoxide poisoning) or the tissues' ability to use the oxygen they receive (cyanide poisoning). Hypoxia forces cells to shift to anaerobic fermentation, which may be harmful in two ways: (1) It may produce too little ATP to meet the cell's needs, causing the cell to die from a shortage of usable energy, and (2) hypoxia produces lactic acid, which lowers the pH of the cell, especially in ischemic tissues where blood flow is inadequate to remove the lactic acid from the tissue.

Free Radicals

The destructive potential of *free radicals* was discussed in chapter 2 (see *A&P,* p. 56). Free radicals are produced as a normal, toxic by-product of cell metabolism (especially aerobic respiration) and by exposure to radiation and some chemicals. Free radicals can kill cells in three ways: lipid peroxidation (destruction of unsaturated fatty acids), protein fragmentation, and DNA alterations. Of the three, *lipid peroxidation* is probably the most destructive. When the free radical (usually OH•) reacts with the double bond of an unsaturated fatty acid, the reaction generates peroxide. The peroxide then initiates a series of reactions that damage the plasma membrane and the membranes of the cell's organelles, killing the cell. In *protein fragmentation,* peroxide reacts with specific amino acid side chains and irreversibly disrupts the secondary and tertiary structures of the protein, leading to fragmentation. When peroxides interact with many parts of the DNA molecule, *DNA alterations* are induced that destroy the cell's genetic functions. Fortunately, the body has several mechanisms that limit free radical damage, as discussed in your textbook.

Chemicals

Chemical injury occurs when a toxic substance damages the various membranes of the cell. This increases membrane permeability and causes the cell and its organelles, especially the mitochondria and endoplasmic reticulum, to swell with water. ATP synthesis and protein synthesis thus break down, gravely compromising the cell's homeostasis. If unchecked, damage to the lysosomes also occurs, and the cell undergoes *autolysis* (self-digestion).

Nutritional Imbalances

Normal cellular function depends on the availability of adequate nutrients such as amino acids, glucose, lipids, minerals, and vitamins. Either excessive or insufficient nutrient levels can harm cells. Usually our diets and the actions of the digestive and cardiovascular systems ensure that cells are appropriately nourished. Some specific cellular effects of nutritional deficiencies are discussed in chapter 26.

Temperature Extremes

Temperature extremes are among the physical factors that can cause cellular injury. Freezing or chilling cells induces *hypothermic injury.* As ice crystals form and melt within cells, they damage the plasma membrane and cause an inflow of sodium ions and water, thus disrupting the cell's osmotic balance. Depending on the rate of chilling, hypothermia can also cause vasoconstriction and ischemia, or it can paralyze the blood vessels in a dilated state, increasing flow and producing severe tissue swelling. In frostbite, blood clotting in the damaged vessels leads to ischemia and gangrene. *Hyperthermia* (excessive body temperature, either because of sun exposure or fever) damages cellular metabolism by speeding up some enzymatic reactions more than others. This causes metabolic pathways to get out of synchrony with each other and leads to derangement of the cell's homeostasis. Extreme heat "cooks" the cell's structural proteins and enzymes, thus causing the immediate tissue death characteristic of burns (see *A&P,* p. 223).

Some Signs and Effects of Cellular Injury

Injured, failing cells are often unable to maintain normal plasma membrane function, so they accumulate fluid. The waterlogged cells have a light-staining, "vacuolated" appearance *(hydropic degeneration).* In addition, failing cells are often unable to metabolize fatty acids, and thus they accumulate lipids in the cytoplasm *(fatty change).* Fatty change is commonly seen in the liver cells of alcoholics, where gross examination (nonmicroscopic inspection) shows a yellowish, greasy-looking **fatty liver.** This can progress to an accumulation of fibrous scar tissue, giving the liver a lumpy surface, a state called **cirrhosis.** Intracellular lipid accumulation is also characteristic of Tay-Sachs and Gaucher diseases (see chapter 4 of this manual).

Other substances, such as pigments, calcium salts, and urate, may also infiltrate damaged cells. Glycogen accumulation is characteristic of Pompe disease and type II glycogen-storage disease (see chapter 4). Pigment accumulation can result from causes as diverse as tattooing, sun exposure, diets high in carotene, or bilirubin accumulation in liver failure.

Dystrophic calcification is the accumulation of calcium salts in dead or dying cells. This is seen in chronic tuberculosis, advanced atherosclerosis, and injured heart valves. Even normal cells accumulate calcium if the concentration of Ca^{2+} in the blood is abnormally high. This is known as **metastatic calcification,** and it can result from vitamin D excess, Addison disease, or bone tumors that decalcify the bones and raise the blood Ca^{2+} level.

Uric acid (or urate, the ionized form) is produced by the breakdown of purines, a component of ATP and nucleic acids. Tissues begin to accumulate crystals of sodium urate if the serum urate concentration rises too high (above 4–5 mg/dL, depending on sex). This triggers an inflammatory condition called **gout,** which may include extremely painful joint inflammation *(gouty arthritis)* and the appearance of small, white nodules *(tophi)* under the skin. Gout can result from abnormal purine metabolism or from insufficient urate excretion by the kidneys. About half of all attacks of gouty arthritis occur in the great toe, and the other half are distributed mainly among the heel, ankle, instep, knee, elbow, and wrist. Gout is at least partially hereditary, and about 95% of its victims are men.

Methods in Histopathology

A **histopathologist** is a physician (M.D.) specialized in recognizing pathological changes in the microscopic appearance of tissues and cells and making diagnoses based on this appearance. A histopathologist is assisted by a staff that may include a pathologist's assistant, histotechnologists, and histotechnicians. A **histotechnologist** is a person who specializes in preparing histological specimens for microscopic examination by the pathologist. A histotechnologist generally must have a baccalaureate degree (preferably in science) and a year of on-the-job training. **Histotechnicians,** who assist histotechnologists, typically have a 2-year diploma or associate degree.

In a hospital setting, histopathology laboratories receive tissues and organs from operating and delivery rooms. Histotechnologists and pathologists often must prepare and examine the tissue and report their findings while the patient is still on the operating table so that the surgeon can determine the appropriate course of action.

Before a tissue can be examined microscopically, it must be sliced into *tissue sections* thin enough to see through—typically about 7 μm thick, which is less than the thickness of many cells. This is done with an instrument called a **microtome,** similar in principle to a butcher's meat slicer but capable of far greater precision. The microtome advances the tissue by fine degrees and shaves off a thin slice each time the tissue passes over the blade of an ultrasharp knife. However, tissues fresh from the body cannot be cut on the microtome because they are too soft; it would be like trying to slice a fresh loaf of bread with a very dull knife. The blade would squash the tissue before it cut it and cause so much distortion in the tissue's structure that the specimen would be useless for diagnostic purposes. Normally, the tissue has to be embedded in a supportive block of paraffin before it is sectioned. A problem with this is that tissue is mostly water, and water and paraffin do not mix. Therefore, some preparation is needed before the embedding process.

The traditional steps of tissue preparation carried out by a histotechnologist are:

- **Fixation** The tissue is cut into small pieces and immersed in formalin or another chemical *fixative,* which prevents decay and somewhat hardens the tissue. Good preservation typically requires at least overnight immersion in the fixative.
- **Dehydration** The specimen is immersed in a series of ethanol baths of increasing concentration, ending with two baths of 100% ethanol, to remove all the water.
- **Clearing** The tissue is treated with a *clearant* such as xylol, clove oil, or wintergreen oil to remove the ethanol. The clearant mixes with paraffin.
- **Embedding** The tissue is immersed in melted paraffin, which infiltrates the tissue and replaces the clearant. The paraffin is then allowed to cool slowly and harden into a solid block.
- **Sectioning** The paraffin block is mounted on the microtome, and a series of very thin slices are cut from it.
- **Mounting** The paraffin-embedded slices are mounted on microscope slides with an adhesive such as albumin. The paraffin is then dissolved out, leaving only a ghostly white or gray tissue section on the slide.
- **Rehydration** In preparation for staining with water-based dyes, the paraffin is dissolved away with a solvent, and the slide is then immersed in a series of progressively lower concentrations of ethanol to rehydrate it.
- **Staining** The slide is immersed in one or more dye solutions that stain different components of the tissue different colors, bringing out the contrast necessary for visual examination. The most commonly used stains are a pair called *hematoxylin and eosin (H&E).* Hematoxylin stains cell nuclei blue to violet, and eosin stains the cytoplasm pink. Different stains are used for specialized purposes such as staining blood, fat, or collagen.
- **Clearing** The stained tissue is treated with a clearing agent again to render it transparent, similar to the way a drop of oil on a piece of paper lets light shine through.
- **Coverslipping** If a specimen is to be kept permanently, a glass coverslip may be applied to the slide with an adhesive. This is not necessary for routine diagnostic work.

Specimen preparation can require several hours to days, because the fixation, clearing, dehydration, infiltration, and rehydration steps must be carried out slowly and carefully to ensure good, useful preparations and to minimize distortion of the tissue. However, if a patient is on the operating table and a surgical decision must be made, there isn't time for this classic technique. The procedure can then be accelerated by freezing the tissue, typically at temperatures of –20°C to –50°C, in a chamber called a **cryostat.** Since freezing hardens the tissue, it takes the place of paraffin embedding—and if paraffin is not going to be used, dehydration, clearing, and rehydration (the most time-consuming steps) are not necessary. The frozen tissue is sectioned with a **freezing microtome** and then thawed and stained.

Once the tissue specimens are prepared, they are examined by a pathologist. In some cases, when specialized methods of diagnosis are needed (such as an assay for estrogen receptors as in the case study that follows), tissue specimens may be sent out to a regional laboratory for preparation and diagnosis. This is routine in the grading of Pap smears, for example.

Case Study 6 Breast Tumors—A Day in the Histopathology Lab

Frances is a board-certified histotechnologist who works at a large urban hospital. Her job consists mainly of receiving tissues and organs from surgeons and preparing them so they can be examined by the pathologist, Dr. Griffin, for diagnostic purposes.

One day, Frances receives specimens from two patients—Ms. Bennett and Ms. Malcolm—who have presented with lumps in their breasts. The oncologist has excised the lumps and sent them to the histology lab. He needs to know whether the lumps are benign or malignant, and if malignant, whether they are local or invasive so that he can decide whether to biopsy the axillary lymph nodes for possible metastatic cancer. The patients remain in the operating room while the specimens are biopsied, so the frozen-section method is used to speed up the diagnostic process.

Ms. Bennett, the first patient, is a single, 28-year-old graduate student. She had not been in the practice of doing breast self-examination (BSE), but was inspired by a newspaper article to start. To her dismay, while doing a BSE in the shower recently, she felt a bean-sized lump in the lower outer quadrant of her right breast. She has visited her physician in deep fear that she has breast cancer.

Dr. Griffin invites Frances to look at the specimen through a dual-head microscope, pointing to a circular, well-circumscribed mass surrounded by a layer of dense, irregular, fibrous connective tissue. Around it are the adipose and areolar tissue typical of normal breast tissue. The mass is composed of loose fibrous tissue and cells that resemble those in the mammary ducts. On the basis of this examination, Dr. Griffin tells Frances that she is diagnosing this as a benign tumor called a **fibroadenoma**, common in young women, and she phones Ms. Bennett's oncologist with the information.

Ms. Malcolm, the other patient, is a 48-year-old attorney and mother of four who has always been in the habit of doing BSEs. She had a baseline mammogram when she was 36 and has been getting mammograms every 2 years throughout her 40s, partly because both her maternal grandmother and her older sister have had breast cancer. Ms. Malcolm has never detected a lump in her own breasts, and yet when she had her last biennial mammogram, a dense mass about 12 mm in diameter appeared in the upper left quadrant of one breast.

Dr. Griffin shows Frances the slides of this biopsy. Mixed among the normal fibrous tissue and adipocytes of the breast are several irregularly shaped clusters of cells and dense fibrous tissue. The cells resemble epithelium, but look immature and are not arranged in epithelial sheets. They are not surrounded by a fibrous capsule, but form tonguelike projections that extend into the surrounding fat and loose fibrous tissue of the breast. Speaking into a voice recorder, Dr. Griffin scans the slide and describes the cells as **pleomorphic, hyperchromatic,** and anaplastic (see "Selected Clinical Terms" for definitions). Many of the cells that Dr. Griffin points out exhibit **mitotic figures.** A small area of necrotic tissue appears in the center of the lesion. Dr. Griffin, looking a little downcast, says "Well, this one looks malignant—an **invasive ductal carcinoma,** I'd say." She adds that they will send the specimen off to a lab to have a mitotic index and immunoperoxidase assay performed.

Some breast cancers are estrogen-sensitive, and the immunoperoxidase assay is a way of determining whether the malignant cells have estrogen receptors. Immunoperoxidase is a monoclonal antibody (see *A&P,* clinical insight 21.2, p. 826) that binds to estrogen receptors in cell nuclei. The slides are then washed with a peroxide reagent. If any antibody is bound to nuclear receptors, it reacts with the reagent to produce a brown precipitate. Dr. Griffin shows Frances some older slides so that she can see what a positive assay looks like. Most of the tissue is gray in color, but the cell nuclei are brown. "That means this was an estrogen-sensitive tumor," Dr. Griffin explains. "This is actually good news. It

gives Ms. Malcolm a better prognosis, because her cancer may respond to tamoxifen therapy."

Based on this case study and other information in this chapter, answer the following questions.

1. What finding especially suggests that Ms. Bennett's tumor is benign? (See *A&P*, chapter 5, p. 156, to review the characteristics of benign and malignant tumors.)
2. Why might Ms. Malcolm's doctor recommend biopsy of the axillary lymph nodes?
3. Why would the mitotic index be relevant to a diagnosis of breast cancer?
4. Tamoxifen blocks estrogen receptors so that estrogen cannot bind to them. How could tamoxifen help in the treatment of certain forms of breast cancer?
5. Considering that X rays are known to induce mutations and that mutations can cause cancer, why are women in their 40s and beyond advised to have routine mammograms?
6. Explain why prolonged anaerobic fermentation could cause the enzymes of a cell to become increasingly dysfunctional and eventually lead to a shutdown of the cell's metabolic pathways.
7. What similarity might you expect in the mechanisms of cell injury seen in hypothermic injury and dystrophic calcification? Explain why both disorders may have similar effects on a cell.
8. Mechanical stress or trauma often triggers the symptoms of gouty arthritis in susceptible people. In view of this, explain why gouty arthritis affects the great toe more commonly than other joints.
9. Patients with gout are often advised to drink 3 liters of water daily. Why do you think this would help relieve their symptoms?
10. If you were using conventional paraffin-based histotechnique, but staining the tissue with an alcohol-based stain instead of a water-based stain, what step in the preparation of the slide could you omit?

Selected Clinical Terms

fibroadenoma A benign neoplasm, common in the breast, composed of fibrous connective tissue, proliferating fibroblasts, and anaplastic cells derived from the ductal epithelium of the mammary gland.

histotechnologist A specialist in the preparation of tissue specimens for microscopic examination.

hyperchromatic Staining more intensely than normal with histologic stains.

invasive ductal carcinoma An advanced form of breast cancer in which malignant cells of the mammary ducts have broken through the basement membrane of the duct epithelium and invaded the connective tissue stroma of the breast. This has the worst prognosis of any form of breast cancer.

microtome An instrument that cuts tissue specimens into extremely thin slices suitable for staining and microscopic examination.

mitotic figures Darkly staining aggregates of condensed chromosomes seen in stained cells, indicating that mitosis was underway when the cell was fixed.

mitotic index A count of the percentage of cells in a given area of tissue that exhibit mitotic figures. An abnormally high mitotic index indicates *neoplasia* (the development of a tumor).

pleomorphic Variable in size and shape.