





FIGURE 1.1 • Hematoxylin and eosin (H&E) staining. This series of specimens from the pancreas are serial (adjacent) sections that demonstrate the effect of hematoxylin and eosin used alone and hematoxylin and eosin used in combination. a. This photomicrograph reveals the staining with hematoxylin only. Although there is a general overall staining of the specimen, those components and structures that have a high affinity for the dye are most heavily stained—for example, the nuclear DNA and areas of the cell containing cytoplasmic RNA. b. In this photomicrograph, eosin, the counterstain, likewise has an overall staining effect when used alone. Note, however, that the nuclei are less conspicuous than in the specimen stained with hematoxylin alone. After the specimen is stained with hematoxylin and then prepared for staining with eosin in alcohol solution, the hematoxylin that is not lightly bound is lost, and the eosin then stains those components to which it has a high affinity. c. This photomicrograph reveals the combined staining effect of H&E. × 480.

containing heavy metals that bind to the phospholipids, such as permanganate and osmium, are used (Folder I.I). The routine use of osmium betroxide as a fixative for electron microscopy is the primary reason for the excellent preservation of membranes in electron micrographs.

Other Staining Procedures

Hematoxylin and eosin are used in histology primarily to display structural features.

Despite the merits of H&E staining, the procedure does not adequately reveal certain structural components of histologic sections such as elastic material, reticular fibers, basement membranes, and lipids. When it is desirable to display these components, other staining procedures, most of them selective, can be used. These procedures include the use of orcein and resoccin-fuchsin for elastic material and silver impregnation for reticular fibers and basement membrane material. Although the chemical bases of many staining methods are not always understood, they work. Knowing the components that a procedure reveals is more important than knowing precisely how the procedure works.

HISTOCHEMISTRY AND CYTOCHEMISTRY

Specific chemical procedures can provide information about the function of cells and the extracellular components of tissues.

Histochemical and cytochemical procedures may be based on specific binding of a dye, use of a fluorescent dye-labeled antibody with a particular cell component, or the inherent enzymatic activity of a cell component. In addition, many large molecules found in cells can be localized by the process of autoradiography, in which radioactively tagged precursors of the molecule are incorporated by cells and tissues before fixation. Many of these procedures can be used with both light microscopic and electron microscopic preparations.

Before discussing the chemistry of routine staining and histochemical and cytochemical methods, it is useful to examine briefly the nature of a routinely fixed and embedded section of a specimen.

Chemical Composition of Histologic Samples

The chemical composition of a tissue ready for routine staining differs from living tissue.

The components that remain after fination consist mostly of large molecules that do not readily dissolve, especially after treatment with the fixative. These large molecules, particularly those that react with other large molecules to form macromolecular complexes, are usually preserved in a tissue section. Examples of such large macromolecular complexes include:

- nucleoproteins formed from nucleic acids bound to protein.
- Intracellular cytoskeletal proteins complexed with associated proteins.
- extracellular proteins in large insoluble aggregates, bound to similar molecules by cross-linking of neighboring molecules, as in collagen fiber formation, and

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Table of Contents Atlas Of Normal Histology 6th Edition

- 1. Understanding the eBook Atlas Of Normal Histology 6th Edition
 - The Rise of Digital Reading Atlas Of Normal Histology 6th Edition
 - Advantages of eBooks Over Traditional Books
- 2. Identifying Atlas Of Normal Histology 6th Edition
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
- 3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Atlas Of Normal Histology 6th Edition
 - User-Friendly Interface
- 4. Exploring eBook Recommendations from Atlas Of Normal Histology 6th Edition
 - Personalized Recommendations
 - Atlas Of Normal Histology 6th Edition User Reviews and Ratings
 - Atlas Of Normal Histology 6th Edition and Bestseller Lists
- 5. Accessing Atlas Of Normal Histology 6th Edition Free and Paid eBooks
 - Atlas Of Normal Histology 6th Edition Public Domain eBooks
 - Atlas Of Normal Histology 6th Edition eBook Subscription Services
 - Atlas Of Normal Histology 6th Edition Budget-Friendly Options

- 6. Navigating Atlas Of Normal Histology 6th Edition eBook Formats
 - o ePub, PDF, MOBI, and More
 - Atlas Of Normal Histology 6th Edition Compatibility with Devices
 - Atlas Of Normal Histology 6th Edition Enhanced eBook Features
- 7. Enhancing Your Reading Experience
 - o Adjustable Fonts and Text Sizes of Atlas Of Normal Histology 6th Edition
 - Highlighting and Note-Taking Atlas Of Normal Histology 6th Edition
 - Interactive Elements Atlas Of Normal Histology 6th Edition
- 8. Staying Engaged with Atlas Of Normal Histology 6th Edition
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Atlas Of Normal Histology 6th Edition
- 9. Balancing eBooks and Physical Books Atlas Of Normal Histology 6th Edition
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Atlas Of Normal Histology 6th Edition
- 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
- 11. Cultivating a Reading Routine Atlas Of Normal Histology 6th Edition
 - Setting Reading Goals Atlas Of Normal Histology 6th Edition
 - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Atlas Of Normal Histology 6th Edition
 - Fact-Checking eBook Content of Atlas Of Normal Histology 6th Edition
 - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
- 14. Embracing eBook Trends
 - Integration of Multimedia Elements

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