

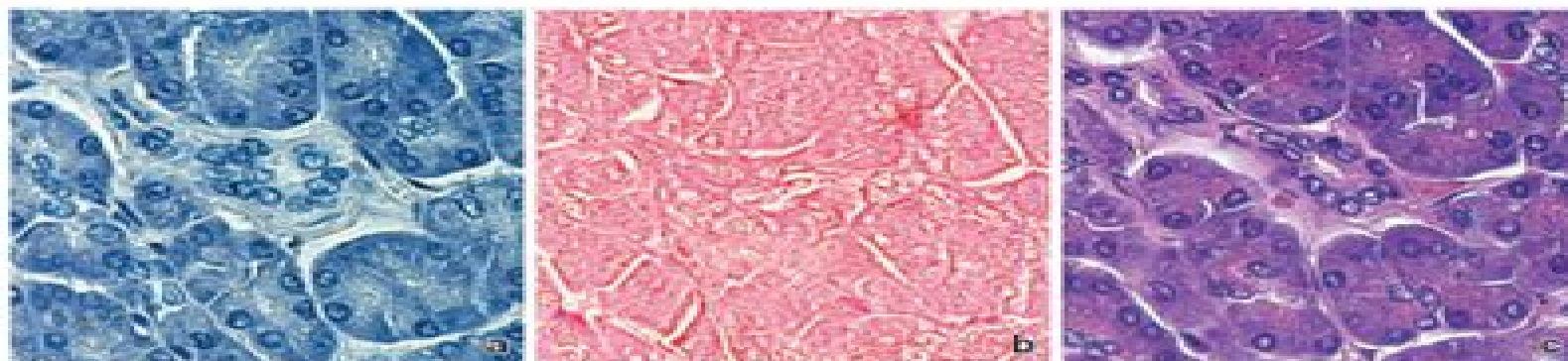


FIGURE 1.1 • Hematoxylin and eosin (H&E) staining. This series of specimens from the pancreas are serial (adjacent) sections that demonstrate the effect of hematoxylin and eosin used alone and hematoxylin and eosin used in combination. **a.** This photomicrograph reveals the staining with hematoxylin 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 tightly 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. $\times 480$.

containing heavy metals that bind to the phospholipids, such as permanganate and osmium, are used (Folder 1.1). The routine use of **osmium tetroxide** 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 resorcin-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 fixation 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 proteins,
- **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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