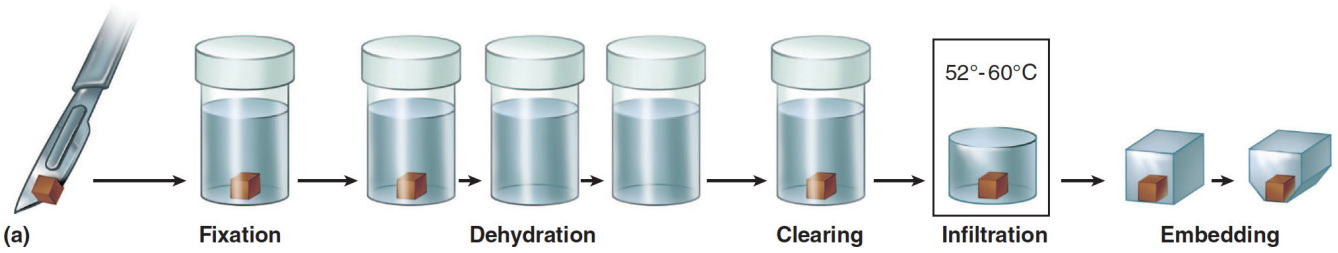
Junqueira's Basic Histology
TEXT AND ATLAS

SIXTEENTH EDITION

Chapter 01. Histology & Its Methods of Study

Mc Graw Hill Education

FIGURE 1–1 Sectioning fixed and embedded tissue.



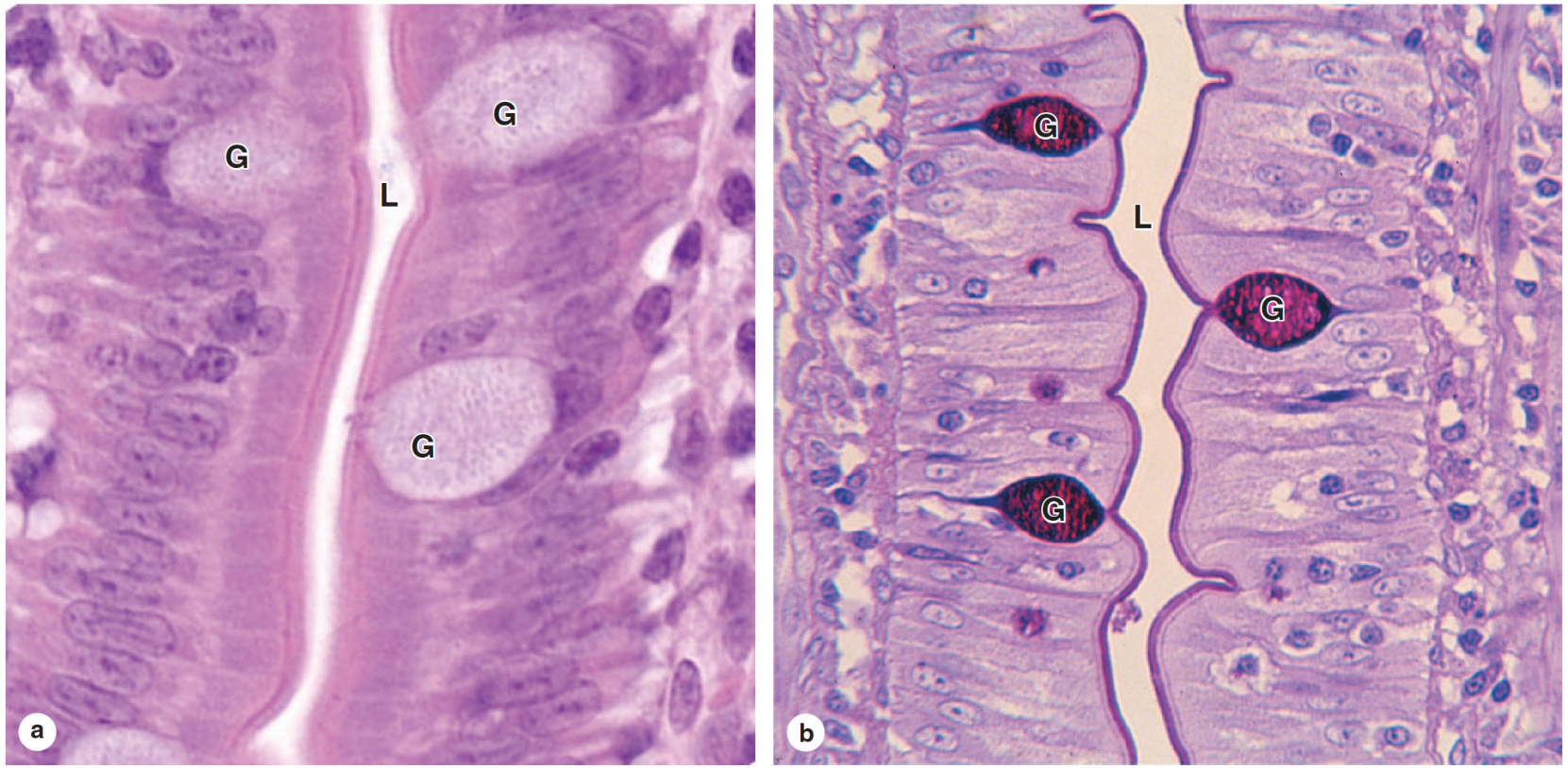
Most tissues studied histologically are prepared as shown, with this sequence of steps **(a)**:

- **Fixation:** Small pieces of tissue are placed in solutions of chemicals that cross-link proteins and inactivate degradative enzymes, which preserve cell and tissue structure.
- **Dehydration:** The tissue is transferred through a series of increasingly concentrated alcohol solutions, ending in 100%, which removes all water.
- **Clearing:** Alcohol is removed in organic solvents in which both alcohol and paraffin are miscible.
- **Infiltration:** The tissue is then placed in melted paraffin until it becomes completely infiltrated with this substance.
- **Embedding:** The paraffin-infiltrated tissue is placed in a small mold with melted paraffin and allowed to harden.
- **Trimming:** The resulting paraffin block is trimmed to expose the tissue for sectioning (slicing) on a microtome.

Similar steps are used in preparing tissue for transmission electron microscopy (TEM), except special fixatives and dehydrating solutions are used with smaller tissue samples and embedding involves epoxy resins which become harder than paraffin to allow very thin sectioning.

(b) A **microtome** is used for sectioning paraffin-embedded tissues for light microscopy. The trimmed tissue specimen is mounted in the paraffin block holder, and each turn of the drive wheel by the histologist advances the holder a controlled distance, generally from 1 to 10 µm. After each forward move, the tissue block passes over the steel knife edge and a section is cut at a thickness equal to the distance the block advanced. The paraffin sections are placed on glass slides and allowed to adhere, deparaffinized, and stained for light microscope study. For TEM, sections less than 1 µm thick are prepared from resin-embedded cells using an ultramicrotome with a glass or diamond knife.

FIGURE 1–2 Hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) staining.



Micrographs of epithelium lining the small intestine, **(a)** stained with H&E, and **(b)** stained with the PAS reaction for glycoproteins. With H&E, basophilic cell nuclei are stained purple, while cytoplasm stains pink. Cell regions with abundant oligosaccharides on glycoproteins, such as the ends of the cells at the lumen (**L**) or the scattered mucus-secreting goblet cells (**G**), are poorly stained. With PAS, however, cell staining is most intense at the lumen, where projecting microvilli have a prominent layer of glycoproteins at the lumen (L) and in the mucin-rich secretory granules of goblet cells. Cell surface glycoproteins and mucin are PAS-positive because of their high content of oligosaccharides and polysaccharides, respectively. The PAS-stained tissue was counterstained with hematoxylin to show the cell nuclei. (a. X400; b. X300)