These questions are designed to solidify and test your knowledge, and to give you representative examples of the types of questions that might appear on tests. Essay type questions generally require **at most** one or two sentences to be answered correctly. On a real exam you would be supplied any necessary formulas or equations. This set of questions will grow as the class progresses.

II. THE SPECIMEN (Section A: BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES)

1. Besides providing a base to support specimens in the microscope, what properties of a support film are important when studying biological specimens? Explain.

2. Carbons films are often used as support films when high-resolution imaging is needed to study a biological specimen. Give three reasons why carbon films make good support films.

3. What are the four important factors ('obstacles') that need to be considered when preparing biological specimens for transmission electron microscopy? Briefly explain.

4. Compare and differentiate the following specimen preparation techniques with respect to the mechanisms used to enhance contrast and the ability to protect against dehydration damage.

   a. Thin-sectioning
   b. Negative-staining
   c. Metal shadow casting (unidirectional)
   d. Frozen-hydrated specimens

5. The inherent contrast of biological specimens is usually very low. Why is this? Briefly cite two fundamentally different ways in which microscopists increase contrast in these specimens to enable fine details to be seen. To receive full credit, make sure the ways you propose are in fact fundamentally different.

6. In each of the following, circle the terms that make each statement correct.

   Thin sectioned material is usually stained after sectioning to enhance (**aperture / interference / absorption**) contrast in the transmission electron microscope. Staining in this way is mainly considered (**negative / positive / neutral**).

   Negative staining is particularly useful for examining (**cells / frozen specimens / particulate samples / sectioned material / freeze-fractured material**).

   Specimen features in frozen-hydrated material appear with (**positive / negative**) contrast because the biological material generally scatters electrons (**less than / more than / the same as**) the surrounding matrix of vitrified water.

7. Pretend, for example, that you have been given a purified preparation of mitochondrial membranes (the actual type of membrane is irrelevant to the question), and would like to learn more about their overall morphology and the arrangement of protein molecules in the lipid bilayer. Describe two different specimen preparation techniques that might be used to study the membrane structure in the TEM and briefly discuss what type of information you might obtain from each method with regard to the structure.

8. Describe two major problems encountered in obtaining satisfactory thin sections of biological specimens. What precautions, if any, can be taken to reduce or eliminate these problems?

9. Fixation with glutaraldehyde preserves what major component of cells /tissues?

10. Why is it usual practice to fix specimens for thin sectioning with more than one fixative?

11. What is the range in thickness of a typical tissue thin section prepared for transmission electron microscopy? Circle the single best answer.

    50-90 nm

    0.1-0.2 cm

    3-5 nm

    0.1-1.0 μm

12. The following list describes some of the characteristics that an ideal negative stain should possess. Briefly discuss why each is important.

    a. High density
    b. High solubility
    c. High melting/boiling points
    d. Dries amorphously
    e. Non-reactive
    f. Protects against dehydration

13. What advantages/disadvantages does the negative staining technique have compared to:

    1) Thin sectioning
    2) Frozen-hydrated samples
    3) Metal shadow cast samples
    4) Freeze-fractured and etched samples

14. Even when they are negatively stained, most biological specimens are to some extent positively stained. Why is this?

15. What is the main limitation to resolution in metal-shadowed biological material?

16. The native contrast of unstained specimens is relatively weak. What technique is used to help overcome this problem with frozen-hydrated specimens?

17. How can the microscopist judge whether or not vitrification of the aqueous solvent has been achieved in preparing frozen-hydrated biological samples?

18. What is the main cause for the decreased radiation sensitivity of frozen-hydrated specimens when compared with unstained specimens at room temperature?

19. Which of the following is NOT an advantage of vitrifying particulate samples for electron microscopy?

a. Preserves the sample to atomic resolution
b. Provides a 2-5 fold reduction in radiation damage
c. Contrast in the image is due to that of the sample and not of a heavy metal stain
d. The technique is relatively simple to perform and easy to master

20. Biological specimens prepared for cryo-microscopy must be vitrified, which requires the cooling procedure to be carried out very quickly. Why is this necessary? What material is used to vitrify specimens?

21. Compare and contrast freeze-drying and frozen-hydrated specimen preparation methods. Identify some advantages and disadvantages of each.

II. THE SPECIMEN (Section B: RADIATION EFFECTS)

1. Identify and discuss three of the many ways that are available to reduce radiation damage to biological specimens.

2. Why is it that, under normal operating conditions with the TEM, the TOTAL DOSE rather than the DOSE RATE is a more important factor in determining the overall damage to biological specimens?

3. Transmission electron microscopy of biological specimens has been called "Microtephroscopy" or, translated, "the microscopy of ashes". What does this mean? Do the ashes bear any resemblance to native structures? Why or why not?

4. It is the (**unscattered / elastically scattered / inelastically**) scattered electrons that lead to radiation damage in biological specimens. (Circle the correct answer and **explain**).

5. Chemical modification of biological specimens, induced by the incident electron beam, is not eliminated even at irradiation levels as low as 50 e/nm^2 at 1 MeV. **(TRUE / FALSE)**

6. A primary effect of the electron beam on the specimen is to heat it even when low dose techniques are used to image beam sensitive specimens. **(TRUE / FALSE)**

7. To minimize specimen beam damage to biological TEM samples it is best to (**increase / decrease**) the microscope accelerating voltage and focus condenser lens 2 (**at / above**) the specimen plane.

8. Thermal effects caused by the interaction of the electron beam with the specimen directly lead to the fragmentation (scission) of chemical bonds and subsequent mass loss. **(TRUE / FALSE)**

9. Chemical cross-linking caused by the interaction of the electron beam with the specimen generally predominates over scission or chemical bond breaking and is the reason that specimens do not totally disappear in the beam with prolonged exposure. **(TRUE / FALSE)**

10. Increasing the accelerating voltage will result in increased beam damage to your specimen. **(TRUE / FALSE)**