Answers

2.1 Methods in Histology

1. a + b + c + d + e +
   Fixatives are used to stop the dynamic processes in cells, render cell components insoluble, denature proteins, prevent and cell autolysis and preserve cell structure ideally in a condition close to that found in living cells.

2. a + b – c + d + e –
   Histological stains are used to provide contrast and differentiate between specific components of tissues and between different tissues

3. a + b – c + d – e +
   Hematoxylin is a basic stain that stains nuclei
   blue to blue-black. It also stains acidic components of cytoplasm and will thus give a blue coloration, for example, to areas of ribonucleoproteins such as are found in concentrations of lamellae of rough (granular) endoplasmic reticulum.

4. a + b – c – d – e –
   Hamatoxylin is a basic dye used in routine staining that reacts preferentially with nuclei acids and acidic groups of proteins. The color achieved is usually a shade of blue or blue-black depending on the type of hematoxylin and the technique used.

5. a – b + c – d + e –

6. a – b – c + d + e +
   Vital dyes are used only on unfixed living materials. They must be particulate, colored and non-toxic to cells and are widely used to demonstrate phenomena such as phagocytosis. Vital dyes in common use include: neutral red, Janus green and trypan blue.

7. a + b + c + d + e +
   There are several methods used to increase contrast in histological preparations and these include the use of special microscopic techniques such as phase contrast microscopy, interference microscopy, polarizing microscopy and dark-ground illumination. All these microscopical methods have specific advantages and disadvantages. Contrast can also be increased using appropriate filters or by staining the tissue

8. a + b + c + d – e –
   Living cells are regularly examined by phase contrast microscopy, interference microscopy and fluorescence microscopy. Living cells cannot be examined in electron microscopes as these operate only under high vacuum, which necessitates the prior dehydration of the material.

9. a + b + c + d + e +
Biological living tissue can be examined by phase contrast microscopy, polarizing microscopy and fluorescence and microscopy without initial fixation. Similarly fixation is not necessarily done with frozen sections or in freeze-fracture techniques

10. a – b + c + d + e +

11. a + b + c + d + e +
Fluorescence microscopy requires the use of special microscopes. Usually these have an ultraviolet light source and the microscope incorporates special filters to protect the eyes. Typically, quartz glass is used to eliminate autofluorescence that might be found in ordinary glass. An many objects that fluoresce are weak, the microscope is usually operated in a darkened room. Fluorescence microscopy is widely used in laboratory diagnosis to detect the localization of specific antigents or malignant cells, which may show a different fluorescence form normal cells. Tubercle bacilli can be readily detected after treatment with a specific fluorochrome. Tetracycline, the antibiotic, is incorporated into bone and can be detected by its fluorescence. By using tetracycline labels at specific intervalsthe rate of bone deposition in compact bone can be measured.

12. a – b + c + d e +
Total preparations, as the name suggests, are not sectioned, but are complete structures that must be thin and transparent. They retain the spatial relationships of components of the tissue. Because these preparations are thicker and more irregular than normal sections, the microscope requires continuous refocusing in order to visualize the various components, which are situated at different levels of focus. The number of cases in which total preparations can be made is fairly limited. A typical example includes the transparent areas of the omentum, which ca be used to demonstrate simple squamous epithelium, loose (areolar) connective tissue, the blood capillary network and fat deposits.

13. a + b + c + d + e +
Histological preparations not needing embedding include total preparations, frozen sections, squashes, smears and exfoliative cytology in its various forms including biopsy scrapings, for example, from the vagina or cervix uteri.

14. a + b + c + d + e +
Frozen sections have advantages over typical wax-embedded material in that processing is rapid. This is invaluable in operating theaters when the surgeon may need a rapid opinion from the pathologist as to whether a growth is malignment and it to proceed with the surgery. As frozen sections cause minimal destruction of enzymatic activity and need not necessarily be fixed, they are widely used in the histochemical and cytochemical localization of enzymatic activity. Frozen

sectioning causes minimal diffusion of small molecules and minimal extraction of lipids.

15. a – b – c – d + e –
Wax-embedded sections for normal
light (optical) microscopy are cut on microtomes usually within the range of 5-10*u*m.

16. a – b – c + d – e –
The effective resolution obtainable by light microscopy is limited by the physical characteristics of light. The effective resolution is about 0.25*u*m.

17. a + b – c +d – e +
Mitochondria, nucleoli and lipid droplets can all be seen using light microscopic techniques.

18. a – b – c + d – e –
The greatest microscopical resolution of components of living cells is obtained using interference microscopy, which has advantages over normal phase contrast microscopy in that no 'phase halo' is present round objects and the various components of cells are 'colored' differently. This can be used in the quantitative determination of such things as the dry mass of the living cells, cell thickness and volume. The basic principle underlying all such determinations is the fact that there is a direct relationship between the concentrations of a solution and its refractive index.

19. a + b + c – d – e –
Phase contrast microscopes make use of annuli in the condenser, which must be brought into co-ordination with the Zernike (retardation) phase plate in the objective lenses. A different substage annulus in usually needed for each objective phase plate.

20. a + b + c + d + e +

21. a + b – c + d – e +
The resolving power of light microscopes is limited by the wavelength of light used. The shorter the wavelength, the better the resolution. The numerical aperture (NA) of objective lenses helps determine their resolving power. NA is dependent on the refractive index of the medium in which the lens is found (air, oil, water) according to the formula:

$$NA = n \sin x$$

Where n is the refractive index of the medium and
x is half the angle of aperature.

$$\text{Resolving power} = \frac{0.61\lambda}{NA}$$

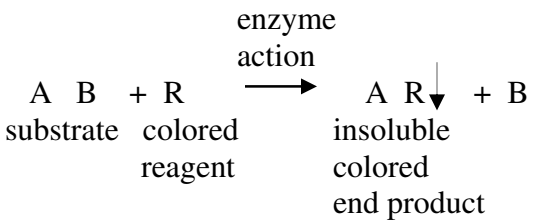
Where λ is the wavelength is light.
Resolution is thus improved by using lenses with a high NA. The NA of oil immersion lenses is greater than that of dry lenses. If optimum resolution is to be achieved the condenser lenses should have a similar NA to that of the objective lenses. Magnification is not a function of resolution. One can magnify objects indefinitely without an increase in resolution in much the same way one can examine a newspaper photograph with a magnifying glass without necessarily improving one's ability to resolve more detail. In practice with light microscopes no more detail is seen if total magnification exceeds 1000 x the NA of the objective lens, which means that the maximum useful magnification of light microscope is about 1400 x. In electron microscopes the wavelength used is very small and the NA of the objective (electromagnetic lenses) is also very small.

22. a – b – c + d + e –
If the light in an optical microscope that is correctly adjusted is too intense for comfortable viewing one can use a lower wattage light source, or if a rheostat is fitted reduce the light output. In color photography using microscopes, a certain voltage is needed for correct color temperature. If the light is too intense neutral density filters can be used.

23. a – b + c – d – e –
Colored filters can provide additional contrast in stained wax sections and are widely used in black and white photomicrography. Yellow filters give most contrast to blue objects, blue filters to yellow objects and red filters for green objects, whilst green filters are used to enhance contrast in red objects.

24. a – b + c + d + e +
Fixation may be used in a large number of techniques for the histochemical localization of enzymes provided that the fixative does not destroy or denature all the enzymatic activity. For example buffered formaldehyde is frequently used as a fixative. Ideally fixation times should be short, make use of well-buffered solutions and be performed at low temperatures.

25. a + b + c + d + e +
Use of frozen sections is often the only way in which the localization of enzyme activity can be determined. In some cases wax-embedded sections can be used, especially when low melting point waxes are used. The end products of histochemical localization of enzyme activity should be insoluble, colored, particulate and not diffuse from the site of the original enzyme activity. This can be expressed as follow:



26. a + b + c + d + e +

In order to determine the sites of specific enzyme activity, fixatives, if used, should be mild, well buffered and non-coagulating. The incubation media should contain specific substrates and be well buffered to the optimal pH and osmolality. The incubation time should be strictly controlled. Various controls should to taken to ensure that the site of localization is correct and that the technique works. Negative is omitted from the incubation medium. Alternatively controls can be used in which the enzymatic activity of the tissue being examined is destroyed by heat treatment. In some technique enzymatic activity can be enhanced or depressed by pre-treatment with activators or inhibitors, which may be help distinguish between closely related enzymes. Positive controls using pieces of tissue from other organs such as kidney or liver, in which specific enzyme activity is known to be present, can be used to show that the technique in question is reliable. In addition more than one technique can be used to localize the activity of a specific enzyme e.g. alkaline phosphatase activity can be determined using metal-salt replacement technique or azo dye techniques.

27. a – b + c – d – e +

Enzymes that are typically localized in lysomsomes and which can be used as markers for these organelles include: acid phosphatase and aryl sulfatase. All the enzymes of lysosomes fall into the category of acid hydrolases.

28. a – b – c + d – e –

29. a – b + c – d – e –

SI (Systèmé Internationale) Units are now becoming universally used in textbooks and scientific journals. These units are providing conformity throughout the scientific community and have resulted in the abolition and many terms formerly encountered in histology including ‘micron’. The basic prefixes of the SI units in histology are:

10^{-3} = *milli-* represented by m-
10^{-6} = *micro-* represented by µ-
10^{-9} = *nano-* represented by n-

Thus for length we use:

10^{-3} m = mm (*millimeter*)
10^{-6} m = µm (*micrometer*)
10^{-9} m = nm (*nanometer*)

For weight we use:	For liquids we use:
10^{-3}g = mg	10^{-3}1 = m1
10^{-6} g = µg	10^{-6}1 = µ1
10^{-9}g = ng	10^{-9}1 = n1

30. a + b + c + d + e +

Van Gieson's trichrome stain is widely used in histology for the localization of collagen fibers. It contains both iron hematoxylin and picrofuchsin. Although it does not reveal cellular detail well, it does differentiate well between connective tissue and muscle. Collagen fibers are stained red, whereas muscle or cornified epithelium is stained yellow.

31. a + b – c – d – e –

Although all the people in question left their mark on cytology, it was Rudolf Virchow, who in 1855 conceived the idea that every cell is derived from an existing cell (*'Omnis cellula cellula'*).

32. a + b – c + d + e –

As opposed to organelles such as mitochondria, nuclei, endoplasmic reticulum, lysosomes or peroxisomes, there are other cellular constituents called 'inclusions' and these include: crystals, pigment and glycogen.

33. a + b + c + d – e +

Lysosomes are membrane-bound organelles that contain acid hydrolases. Lysosomes are typically active in intracellular digestive processes, though continually more and more functions are being attributed to lysosomes especially in the area of pathology.

34. a + b + c – d + e –

The polarizing microscope can be used to distinguish between birefringent (anisotropic) materials and non-birefringent (isotropic) material. The A-band in striated muscle received the initial A because it has Anisotropic properties as opposed to the I-band, which is *I*sotropic. Birefringence is often manifest when there is a regular periodicity exhibited by molecules in structures. Bone tissue is birefringent because of the orderly-arranged collagen; collagen itself is birefringent. Crystals are also seen to be birefringent when viewed by polarizing microscopy.

35. a + b + c – d + e +

It is possible to obtain considerable improved resolution from material embedded in epoxy resins and cut on ultramicrotomes into sections about 0.5-1 µm thick. These sections are easily and rapidly stained with warm alkaline toluidine blue or similar stains prior to examination by light microscopy. These preparations are commonly referred to by electron microscopists as 'thick' sections. In most cases it is possible to cut ultrathin sections from the same blocks and examine these for correlated transmission electron microscopy.

36. a – b + c – d – e +

37. a – b – c + d + e +

Sections cut for standard transmission electron microscopy are usually about 50-100nm thick. They may be cut on the ultramicrotome with glass or diamond knives. Sections are typically stained with uranl acetate or lead citrate to provide added contrast. Unstained sections usually lack sufficient contrast to allow enough detail to be seen w hen they are viewed in the electron microscope. Osmium tetroxide, in addition to being an effective fixative, has the further advantage of staining the lipid components of cells, including the phospho- lipid of cell membranes.

38. a – b + c + d + e +

39. a + b + c + d + e +

There are many common dyes or stains used in histology that contain fuchsin, such as basic fuchsin or acid fuchsin. Lipofuscin is not to be confused with these, but is a naturally occurring 'pigment' or inclusion typical of aged cells. It is sometimes referred to as 'aging pigment' or 'wear and tear' pigment and is typically found in cells that do not divide after birth such as neurons or muscle. Lipofuscin is especially common in aging cardiac muscle. Lipofuscin is seen in the form of brown colored granules, which may show autofluorescence when excited by ultraviolet illumination. Now adays liopofuscin is widely regarded as a form of secondary lysosome or residual body.