

Answer the following questions :

GROUP (A)
(15 marks)

Question No. 1

Choose the best response to each statement.

1. Periodic acid Schiff (PAS) reaction stain the following except,
 - a. Basement membrane.
 - b. polysaccharides.
 - c. Lipofuchsin
 - d. DNA
 - e. glycogen .
2. Which of the following would be best suited to differentiate collagen fibers from other fibers?
 - a. Wright's stain
 - b. Hematoxylin and eosin stain
 - c. Sudan stain
 - d. Silver impregnation
 - e. Masson's trachoma stain
3. What color do elastic fibers stain with Orcin Elastic stain?
 - a. Orange
 - b. Pink/red
 - c. Blue/black
 - d. Green/blue
4. Which of the following would be best suited to visualize reticular fibers?
 - a. Orcin
 - b. Hematoxylin and eosin stain
 - c. Sudan stain
 - d. Silver impregnation
 - e. Masson's trichrome stain
5. The histochemical reaction which confirms the presence of chemical substance is known as .
 - a. histochemical reaction.
 - b. reversible reaction.
 - c. control reaction
 - d. specific reaction
6. Accumulation of glycogen at one side of fixed cell
 - a. Glycogen flight
 - b. Glycogen distribution in the hepatic lobule
 - d. Glycogen depletion
 - c. Glycogen in basement membrane
7. Which of the following would be best suited to visualize nucleic acids?
 - a. Wright's stain
 - b. PAS stain
 - c. Sudan stain
 - d. Silver impregnation
 - e. Methylene green pylonin
8. A polysaccharide which act as strong reducing agent is,
 - a. glycogen
 - b. ascorbic acid
 - c. mucopolysaccharides
 - d. glycoprotein.
9. A histochemical reaction which is specific for glycogen only is,
 - a. PAS reaction
 - b. Best carmine
 - c. Alcian blue
 - d. Silver nitrate reaction
10. Fibroblasts are secretory cells responsible for the formation of
 - a. Collagen
 - b. Reticulin

c. All the previous d. Non the previous

11. A type of protein can't be demonstrated histochemically.

- a. Histones
- b. Keratin
- c. Elastin d. Collagen
- e. Hemoglobin

12. In Feulgen reaction, DNA digested by using

- a. Hydrochloric acid
- b. Deoxyribonucleases
- c. Diastase
- d. Acetate puffer
- e. Acetate puffer

13. RNA is found in large amounts in reproducing active cells as.....

- a. yeasts
- b. tumor cells
- c. hepatic cells
- d. all the previous

14. Collagenous (White) fibers are.....

- a. strong fibers
- b. branched bundles
- c. peptides fibers
- d. All the previous
- e. non the previous

15. Schiff reagent gives positive purple colour with.....

- a. Aldehyde group.
- b. hydrolyzed deoxyribose sugar
- c. w-hydroxyalaevulinic aldehyde
- d. All the previous

16. High levels of serum lipase activity in body fluids indicate in case of :-

- a. Liver disease.
- b. Vitamin a deficiency.
- c- Diabetes mellitus.
- d- Acute pancreatitis. -

17. The optimal PH of alkaline phosphatases reaction is :-

- a-7
- b- 6.2
- c- 9.4 .
- d- 5

18. In Gomori metal precipitation technique for alkaline phosphates which activator used :-

- a- magnesium.
- b- calcium .
- c- manganese.
- d- ammonium.

19. Cold acetone fixative is better employed for

- a- Phosphatases.
- b- Dehydrogenases.
- c- Glycosidases.
- d- oxidases

20. The final reaction product produce at the site of enzyme activity must be:

- a- invisible and insoluble.
- b- Stable and soluble.
- c- Stable and insoluble.
- d- visible and soluble

21. For demonstration of lipids it better to use

- a- Paraffin section.
- b -Frozen section.
- c- Both a &b.

22. Section must be treated withto from Black ppt. in metal substitution method.

- a-diazoniumsalts
- b- tetrazolium salt
- c-Ammonium sulphide
- d- cobalt sulphide

23. Lipids compounds that found principally in the nervous tissue is.....

- a- Glycolipid.
c-Lipoproteins.
- b. Phospholipids
d-Phosphoproteins.

24. Haemosidrin is.....

- a- a golden brown pigments
c- can identified histochemically by Prussian blue technique
- b- found in liver, spleen and bone marrow
d- all of the above.

25. Which color of the following of Lipofuscin pigments?

- a-yellow brown
c- yellow globules
- b- golden brown
d- greenish blue color

26. Which of the following would be best stain to visualize melanin pigments?

- a. Gmelin method
c. Sudan stain
- b. argentaffin reaction.
d. Schmorl reaction

27. A fixative which is specific for oil red O is.....

- a- Cold alcohol
c- calcium phosphate
- b- formol calcium chlorides
d- all of the above

28. Succinic dehydrogenase enzyme found in great amount in.....

- a- free in the living cells.
c- associated with cell surface.
- b- Associated with large particles.
d- all of the above.

29. Unmasking lipids appear as a result of.....

- a- disappearance of protein membrane
c- transformation into fats
- b- increase of lipid proportion
d- All of the above.

30. PAS reaction gives positive purple colour with.....

- a-Haemosidrin
c- Chromaffin
- b- Lipofuscin.
d- Melanin.

Question No. 2

(5 marks)

Write the scientific term:

1. A cellular structure gives strong PAS reaction. **basement membrane**
2. A cellular material stained by Schiff reagent. **glycogen**
3. The microscope which used in histochemical study . **light microscope**
4. A strong basic nucleoprotein. **histone**
5. A polysaccharide which giving raises positive alcian blue reaction . **mucopolysaccharides**

Question No. 3:

Give an account on TWO only of the following :- (Marks)

A- Intracellular distribution of enzymes.

B - Bile pigments.

C- Gomori method for phosphatases.

A- Intracellular distribution of enzymes.

The concept of the cell as a "sack of enzymes" has given way to recognition of the important significance of spatial arrangement and compartmentalization of enzymes, substrates, and co-factors within the cell. For example, in rat liver cells the glycolytic enzymes are located free in the non-particulate portion of the cytoplasm, whereas the enzymes of the citric acid cycle are contained within the discretely organized mitochondrial particles. Generally speaking, the enzymes may occur.

(1) free in living cells. Examples of these enzymes are pepsin, trypsin, lipase, amylase, esterase, arginase and catalase (the last 3 are found in great amounts in the liver).

(2) associated with large particles in cells, e.g., cytochrome oxidase and succinic dehydrogenase which are accumulated in the mitochondrial membranes, and

(3) Enzymes associated with other cell structures such as the chromosomes, nuclei and cell surface.

The functions of the mitochondrion in energy metabolism and of the ribosomes in protein synthesis were discovered in part by the use of techniques involving differential centrifugation and histochemistry.

The histochemical approach examines the distribution of enzyme activity in a tissue or cell in its native state. Thin (2 to 10 μ) frozen sections of tissue, prepared with the aid of a low-temperature microme, treated with a substrate for a particular enzyme. In regions where enzyme is present the product of the enzyme-catalyzed reaction is formed. If the product is coloured and insoluble, it remains at the site of formation and serves as a marker for the localization of the enzyme.

Certain highly purified enzymes consist of several molecular species. These different forms of the same enzyme have similar catalytic activity, but differ from one another immunologically, chemically, and electrophoretically. The term isozyme has been suggested to describe enzymes which, although they catalyze the reaction are chemically or physically distinct. One example is lactate dehydrogenase of human blood and other tissue. When the amounts of the different isozymes of lactic dehydrogenase in the tissues are measured. It is often found that there is a considerable variation in amounts of other enzymes also vary in different tissues. These variations may prove useful in identifying the organ from which an enzyme in the serum originates, thus improving the diagnostic precision of serum enzyme assays.

Bile pigments :-

These are usually yellow, orange or green. They consist of a number of different pigments, the two main constituents being bilirubin and haematoidin. The pigments appear as golden-brown globules, which can be demonstrated by the gmelin technique. The before mentioned types of pigments (haemoglobin, haemosiderin and bile pigments) are derived from haemoglobin and are often called the haematogenous pigments.

Gomori(1939) calcium phosphate method (metal substitution) :-

In this technique sodium glycerophosphate is used. This is the classical method of Gomori (1939) and Takamatsu (1939) from which numerous variations are derived. The principle is simple. It depends on the deposition of calcium phosphate at sites of enzyme activity when sections are incubated at about 37°C with an organic phosphate ester in the presence of Ca ions (e.g. Ca nitrate), at pH 9 (pH around 9.4 is kept by the sodium veronal buffer). Most of the phosphates require magnesium ions as activators and these are therefore added in the form of small concentrations of magnesium sulphate or chloride. The method can be summarized in the following:

If a section is placed in an incubating solution containing a substrate (i.e., sodium B-glycerophosphate) and calcium ions (i.e., calcium nitrate), plus an activator for the phosphatase (i.e., magnesium chloride), a precipitate of calcium phosphate is formed at the sites of enzyme activity. The alkaline phosphatase liberates phosphate from the sodium B-glycerophosphate and this then combines with calcium ions to form the calcium phosphate, which is treated with 1% cobalt nitrate to produce cobalt phosphate. The section is then washed in dist. H₂O to remove the excess of cobalt nitrate. This section is then treated with dilute ammonium sulphide to form black cobalt sulphide, which is visible under the light microscope. The reactions can be summarized as follows:-

Sodium B-glycerophosphate + Alkaline phosphatase = phosphate ions .

phosphate ions + cobalt ions = cobalt phosphate .

cobalt phosphate + sulphide ions = cobalt sulphide (black fine precipitate).

The incubating medium does not keep and it is advisable to make up fresh solutions immediately before use. The final PH of the solution must be determined by experiment for cryostat sections, and the longest for fixed and paraffin processed material .