Review Article

Enzyme Histochemistry: A Review

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Abstract
Enzyme histochemistry serves as a link between biochemistry and morphology. It is based on metabolization of a substrate provided to a tissue enzyme in its orthotopic localization. Visualization is accomplished with an insoluble dye product. It is a sensitive dynamic technique that mirrors even early metabolic imbalance of a pathological tissue lesion, combined with the advantage of histotopographic enzyme localization. With the advent of immunohistochemistry and DNA-oriented molecular pathology techniques, the potential of enzyme histochemistry currently tends to be under recognized.
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Introduction:
Enzyme are biocatalysts synthesized by living cells, that increase the rate of reactions without themselves being changed in the overall process.
Histochemistry is defined by Pearse as "the identification, localization and quantification in cells and tissues and by chemical or physical tests, of specific substances, reactive groups and enzyme-catalyzed substances." Thus, any chemical procedure that localizes a substances with in cells or tissues for subsequent microscopy is a histochemical technique. In broad sense, Enzyme histochemistry is the science that encompasses immunologic and molecular biologic technique when they are combined with histology.[1-4]

Principles of enzyme histochemistry :
Histochemistry procedures are based on the simple premise that tissues or cells, when placed in a solution chemically react with the solution to produce a colored insoluble end product. The amount and location of the end product can then be evaluated in the context of the cell or tissue.[5,6]

Classical histochemical reactions are generally based on one of the 4 principles:
1.Simple ionic interactions.
2.Reactions of aldehydes with Schiff's reagent or silver compounds.

3.Coupling of aromatic diazonium salts with aromatic residues on protein.
4.Conversion acting on a substrate to form a colored ppt.

Types of histochemical reactions:[1]

1. Simultaneous capture.
2. Post incubation coupling.
3. Self coloured substrate.
4. Intramolecular rearrangement.

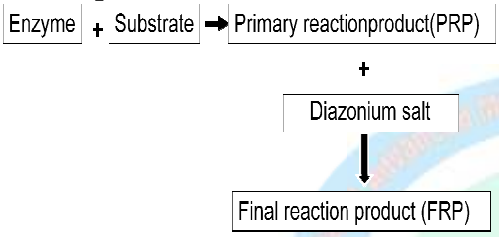
1. Simultaneous capture:

-Most imp. Technique.

Principle:

-Gomori's Metal ppt. Technique
-Azo dye method.

Technique:

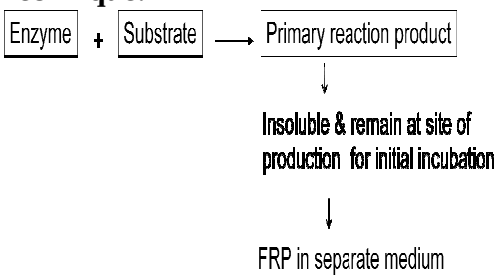


Disadvantage :

1.Diffusion of PRP.
2.Rate of hydrolysis of substrate.
3.Diffusion coefficient of the PRP for the buffer.
4.Rate of coupling of the PRP and diazo salt.
5.Diazo salt and Enzyme same pH .

2. Post incubation coupling:

Technique:



Advantages:

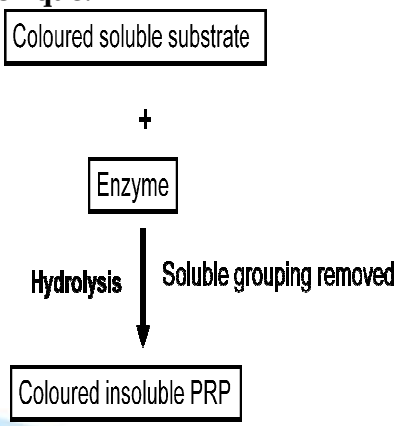
1.Case where long first incubation stage is necessary
2.Optimum pH for enzyme and for diazonium salt separately

Disadvantage :

1.PRP is not completely insoluble
2.Diffusion is always there.

3.Self coloured substrate :

Techique:

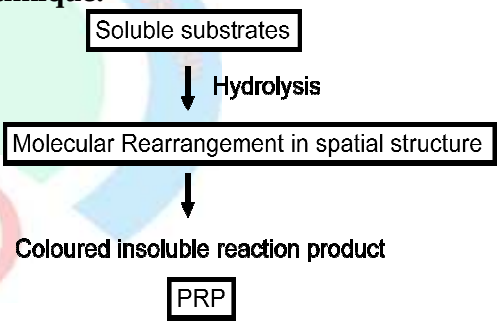


Advantage:

1.Diazonium coupling not required.

4. Intramolecular rearrangement:

Technique:



Diagnostic Applications of enzyme histochemistry:

Enzyme histochemical techniques are not widely applied to surgical and necropsy material for diagnostic purposes, mainly because of the total or partial loss of enzyme activity, which occurs when a tissue is routinely fixed and processed into paraffin.

The current common uses of enzyme histochemistry in surgical histopathology laboratories

1. Skeletal muscle biopsy.
2. Rapid and easy detection of ganglia and nerves in cases of suspected Hirschsprung's disease.

3. Demonstration of specific lactase or sucrase deficiency in jejunal biopsies.
4. Demonstration of mast cells & white cells of the myeloid series.
5. Miscellaneous:

Skeletal muscle biopsy:
Application of enzyme histochemical methods to cryostat sections of unfixed skeletal muscle shows the presence of different fiber types, and changes in the number, size and relative proportions of different fibers which are valuable in establishing the diagnosis.
Muscle biopsy samples are of 2 types:

Open muscle biopsies:
These are received in the laboratory as strips of skeletal muscle, preferably tied at each end to a piece of orange stick.The biopsy sample should be received fresh(unfixed) in the lab as soon as possible after surgical removal.It is transported from operating theater to laboratory wrapped in gauze soaked in normal saline, then squeezed till just damp, to minimize drying. Long delays between surgical removal and freezing can result in unwanted freezing artifact.On arrival, muscle biopsy is cut into suitable block sized pieces(0.5 X 0.5 cm) and oriented so that transverse sections will be cut.[6]

2.Needle biopsy samples: These are taken by a Bergstrom needle and can be quickly and easily obtained from the thigh under L.A,after nicking the skin with a sharp scalpel blade.The biopsy samples are placed on a guaze damped by saline and transferred to the lab as quickly as possible.Under dissecting microscope, biopsies are gently manipulated and trimmed so that the fibers in each are running in the same direction, and a composite block is made of all the samples.Whether the sample is an open biopsy or needle biopsy,it is important that freezing be as rapid as possible,since slow freezing will produce ice crystal artifact which may hamper accurate diagnosis.Although many of the morphological changes in skeletal muscle can be seen on an H & E stain, special methods are necessary to demonstrate some of the structural abnormalities of diagnostic importance, and the most imp. Of these are enzyme histochemical techniques.[6]
Following methods are used rountinely:
Adenosine triphosphate.
NADH diaphorase.
Phosphorylase.

1. Adenosine triphosphatase:
ATPase methods are used in combination to distinguish between type1 and type 2 fibers, and to further subdivide the type 2 fibers into 2A,2B and 2C subtypes. This distinction is diagnostically important since some muscle diseases have characteristic patterns of loss, atrophy or grouping of specific fiber types or subtypes. Some types of structural fiber abnormality (eg. periodic paralysis) are also demonstrated by the ATP-ase methods.

2. NADH diaphorase:
Demonstrates mitochondria and the fine detail of the sarcoplasmic reticulum of the fiber. It is used to detect very minor or early structural abnormality in the sarcoplasmic reticulum network of the fiber, as well as mitochondrial abnormalities. eg. Mitochondrial myopathies.

3. Phosphorylase: Also distinguish between type 1 and 2 fibers but fades very quickly. It is used to exclude **McArdle's disease**, a primary phosphorylase deficiency.

4. Acid phosphatase or non-specific esterase:
To identify macrophages in necrotic fibers and abnormal lysosomal activity in muscle fibers.

5. Cholinesterase :
To highlight atrophic fibers and to demonstrate intramuscular nerve twigs.

Detection of nerves & ganglia in suspected Hirschsprung's disease:
In Hirschsprung's disease in children, a variable segment of the rectum and colon is devoid of ganglionic cells.In the effected segment peristalsis is impossible and the large bowel becomes obstructed.The diagnosis may be suspected clinically and radiologically but requires histological confirmation,usually by the examination of one or more suction biopsy specimens of rectal mucosa and submucosa.The biopsy sample is orientated under dissecting microscope control so that sectioning will include mucosa and submucosa,then snap-frozen at -170 C in isopentane cooled in liquid N_2 and sectioned in a cryostat.Preliminary sections are stained with H & E and the submucosa examined for the presence of ganglia.If sufficient submucosa is present or if no ganglia are seen after examination of a no. of H & E stained levels,then 2 or 3 sections are stained by cholinesterase method to demonstrate the fine nerve twings in the mucosal lamina propria.

Demonstration of specific lactase or sucrase deficiency in jejunal biopsies:
For the assessment of jejunal mucosal biopsies in suspected celiac disease, the specimen can be examined under the dissecting microscope and the presence or absence of villi noted. Paraffin sections stains with H & E are used to assess villous height, gland hyperplasia and intensity of inflammatory cell infiltrate in the lamina propria. Alternatively, the biopsy can be snap frozen, sectioned in a cryostat, and H & E stained for rapid diagnosis.

Advantages:
An alkaline phosphatase method can be applied; Alkaline phosphatase activity resides on the enterocyte surface, is a sensitive marker of structural and functional integrity of the mucosal absorptive cells.This is particularly useful in assessing histological recovery.
Acid phosphatase demonstrates some of the inflammatory cells in lamina propria, and also identifies lysosomal activity in villous enterocytes and glandular crypt epithelial cells.

Demonstation of mast cells & white cells of myeloid series :
Chloroacetate esterase techniques have recently been applied to formalin-fixed paraffin sections to assist in the identification of tissue mast cells and myeloid white cells.
Two methods are suitable:
1. Fast blue RR method: which gives a vivid blue reaction product (particularly intense in mast
cell cytoplasm).
2. Pararosanilin methd: which gives a pinkish-red reaction product.

Miscellaneous:
1. Use of acid phosphatase in the identification of prostate carcinoma.eg.when the tumor is
infiltrating the colon or bladder wall,or in bone metastases.
2. Application of acid and alkaline phosphatase methods to cryostat sections of jejunal mucosal
biopsy specimens.
3. Use of alkaline phosphatase methods in vascular endothelial tumors.

Conclusion:
Enzyme histochemistry serves to detect early metabolic changes in biopsy and autopsy tissue before manifestation on H &E staining or immunohistochemistry. As such, it constitutes a valuable complement to other special techniques, i.e. immune histochemistry and molecular pathology. An exclusive diagnostic domain of enzyme histochemical analysis is the aganglionosis of the distal rectum mucosa, which cannot

reliably be diagnosed by conventional histology alone. Apart from current diagnostic applications in muscle biopsy analysis and the diagnosis of Hirschsprung disease, enzyme histochemistry provides access to a wide range of investigations in experimental pathology and toxicology as a pathophysiological supplement to conventional histology.

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