Cell biology and Histochemistry

(كيمياء الانسجة)

كود

(The theoretical partالجزء النظري)

الفصل الدراسي الاول

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رابط خارجي

- **رؤية جامعة جنوب الوادي 2018 -2023**

التميز في التعليم العالي لبناء تنمية مستدامة في صعيد مصر

- **رسالة جامعة جنوب الوادي 2018 -2023**

تسعي جامعة جنوب الوادي الى إعداد الخريجين لممارسة مهنية وبحثية منافسة إقليميا وعالمياً من خلال قدرة مؤسسية وفاعلية تعليمية جاذبة وداعمة تمكن الطلاب من اكتساب مهارات متطورة، وباحثين قادرين على تطوير تخصصاتهم بتقديم بحوث إبداعية وتطبيقية، وتقديم خدمات مجتمعية وبيئية متميزة تسهم في التنمية المستدامة من خلال بناء شراكات استراتيجية فاعلة وتعزيز القيم الوطنية والهوية الثقافية، والتطوير المستمر لبرامج وكليات الجامعة وإداراتها وتأهيلها للاعتماد، ورفع جاهزية وتنافسية الجامعة واستقلال فرعيها والتوظيف الأمثل للموارد.

- **رؤية كلية العلوم 2018 -2023**

التميز في تعليم العلوم الأساسية والبحث العلمي للمساهمة في التنمية المستدامة

- **رسالة كلية العلوم 2018 -2023**

تقديم تعليم مميز في مجالات العلوم الأساسية وإنتاج بحوث علمية تطبيقية للمساهمة في التنمية المستدامة من خلال إعداد خريجين متميزين طبقا للمعايير الأكاديمية القومية، وتطوير مهارات وقدرات الموارد البشرية، وتوفير خدمات مجتمعية وبيئية تلبي طموحات مجتمع جنوب الوادي، وبناء الشراكات المجتمعية الفاعلة.

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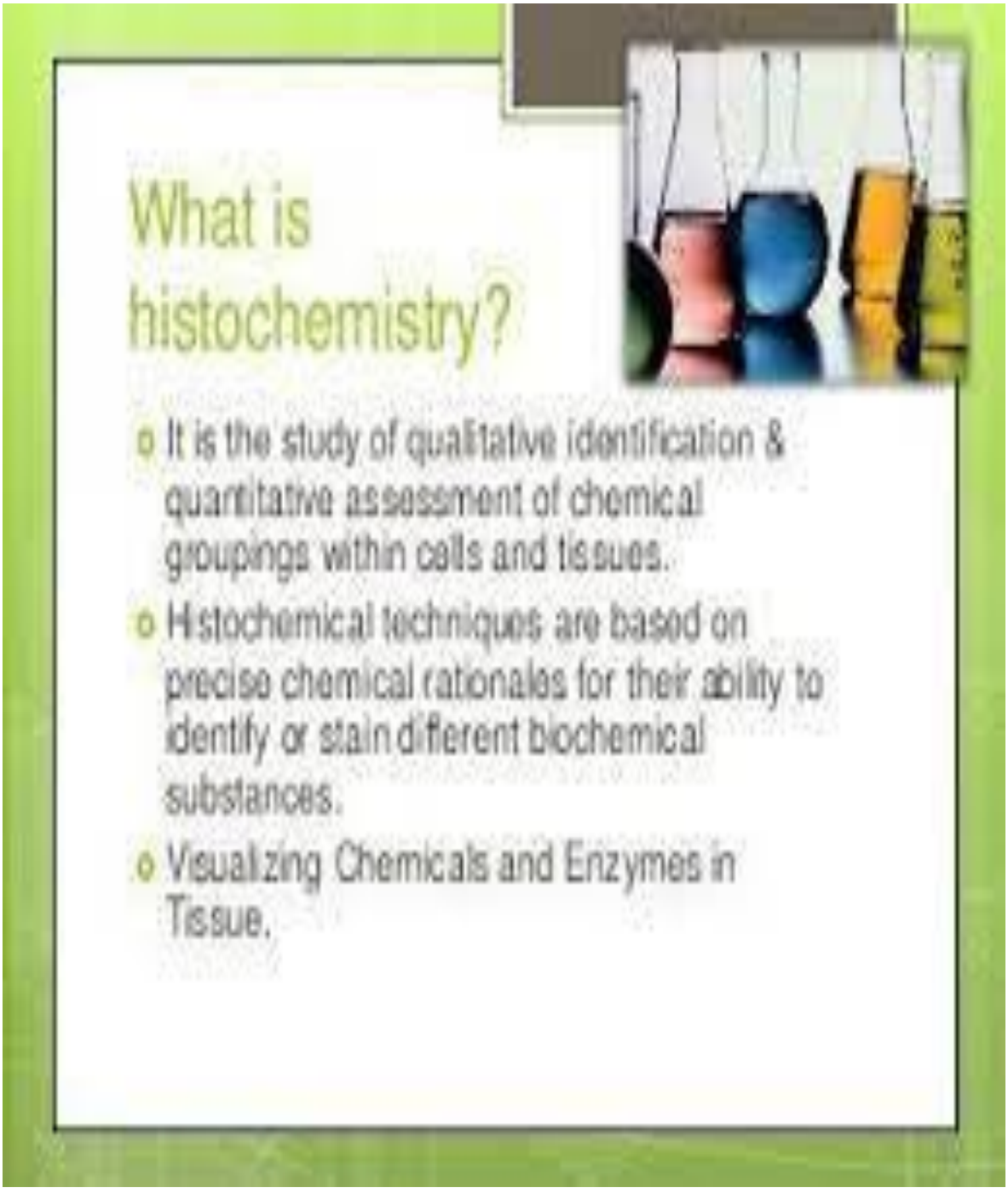
What is histochemistry?
It is the study of qualitative identification & quantitative assessment of chemical groupings within cells and tissues.
Histochemical techniques are based on precise chemical rationales for their ability to identify or stain different biochemical substances.
Visualizing Chemicals and Enzymes in Tissue.

The Histochemistry and Its Aims …

Histochemistry is the science which study the different patterns of chemical components in various cells and tissues in natural (normal) "sites (or places) and also links between these chemical Compounds with different biological functions of cells and tissues

- هو ذلك العلم الذي يهتم بدراسة الأنماط والاشكال المختلفة للمكونات الكيمائية داخل الخلايا والانسجة الطبيعة في اماكنها الطبيعية وكذلك الربط من هذه المكونات الكيميائية والوظائف البيولوجية للخلايا والانسجة

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Aims & Histochemistry

1- Histochemistry enables (allows) the identification and Localization of Chemical components in the tissues.

2- Histochemistry studies the linkage between the Chemical Components that found in the tissue and its functions in the body.

3- Histochemistry used to study the changes which occur. In these components in (unordinary) Conditions and Compare it with in ordinary conditions.

يهدف الي:

1- اظهار وتوضيح وتقدير المكونات الكيميائية داخل الخلايا والانسجة الطبيعة في اماكنها الطبيعية بواسطة الميكروسكوب.
2- الربط بين هذه المكونات الكيميائية والوظائف البيولوجية للخلايا والانسجة.
3- تتبع التغيرات التي تحدث في هذه المكونات الكيميائية في اماكنها الطبيعية في الخلايا والانسجة وذلك تحت الظروف الغير طبيعية(سواء كانت المرضية او التجريبية)، حيث ان كل تغير فيها غالبا ينعكس في صورة تغييرات في تركيب ونشاط الخلايا والانسجة .

- المكونات الكيميائية داخل الخلايا والانسجة تتكونا اساسا من المواد الكيميائية الرئيسية:

Carbohydrates, lipids, proteins, nucleic acid, pigments vitamins, – water, Anions.

The chemistry of the cell structure…?

-Cell is the structural and functional unit in all organisms

- Cells and tissues from structure and function View are Chemical Components and biochemical reactions occur inside them

- Each all consists of 2 main components

1-nucleus 2- Cytoplasm

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Cytoplasm: is divided into 3 parts

1-Cytoplasmic organelles 2- cytoplasmic inclusion 3- Cytoplasmic matrix.

1- cytoplasmic organelles: are divided into two parts(groups)
- membranous cytoplasmic organelles

• such as [cell membrane +[RER, SER] + Golgi apparatus Ly so some + Mitochondria].

2- Non membranous cytoplasmic organelles.

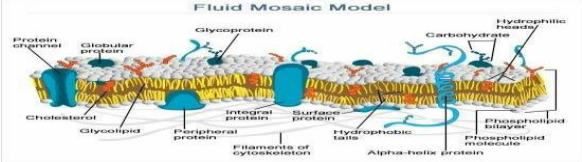
•Such as [Ribosomes+ Cytoskeleton & Micro-tubules flagella + Centrioles + Microfilament]

A- Membranous Cytoplasmic organelles

1- Cell membrane

-Chemical structure: it consists of lipids + Proteins.

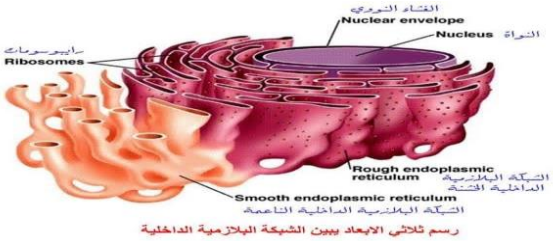
1-lipids: Composed of phospholipids, glycolipids, cholesterol.



2-Protein peripheral integral, Glycoproteins

2- endoplasmic reticulum:

- It consists of tubes connect together
- There is in all cells except RBCS
- There is 2 types of endoplasmic reticulum :-

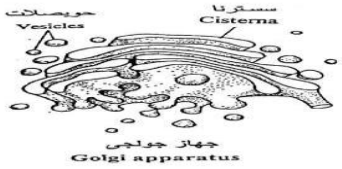


رسم ثلاثي الابعاد يبين الشبكة البلازمية الداخلية

1-R. ER: Rough endorphin reticulum with Ribosomes
2-S.E.R: Smooth endoplasmic vehicular without Ribosomes

3- Golgi apparatus

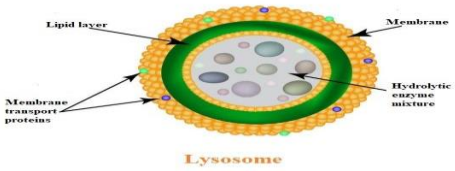
- Composed of saccules separated from each other and has 2 face (Cis + Trans) and there is secretory Vesicles



4- lysosomes

•small secretory Vesicles. And have hydrolytic enzyme

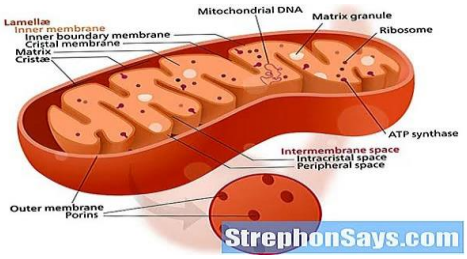
There are 3 types of Lysosomes.



1-primary lissome 2-secondary Lysosome 3-endosome.

5- mitochondria

- It is a membrane-enclosed organelle
- composed of lipids + proteins



*chemical structure :-

1-outer membrane + inner membrane

2-Matrix

* function:

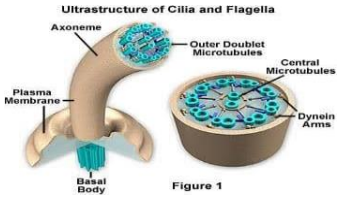
Energy production

*No. Of them increase with increase of activity of all. ADP + Inorganic phosphate every ATP

B- Non membranous cytoplasmic organelles:-

1- Microtubule

- Consist of tubulin protein (a-tubulin B-tubulin) and diameter of microtubules is premed from 13 granules of Protein.
- They Arm Flagella or Olio at for movement



- There are two types at tubulin:_

  1-free in cytoplasm 2-bound which form the flagella

2-Microfilament

•Similar to Microtubules but they are formed from longizaten. Molecules combine each other (actin protein (G- actin + F-actin) to form micro filament.

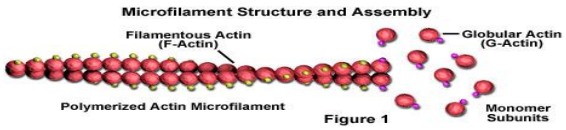
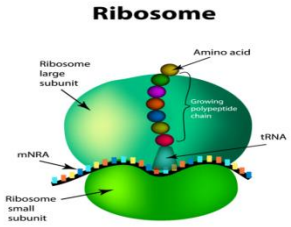


Figure 1

•They are found in muscle fibers

3-Ribosomes

- each ribosome Consist of 2 subunits
  1-small subunit 2- Large subunit



- each subunit consist of FRNA + Protein
- ribosomes covers the rough endoplasmic reticulum(R.E.R)

*chemical structure:-

1-r-RNA → synthesized in nucleus

2-prtern→ synthesized in cytoplasm

*function

-protein synthesis

2-cytoplasmic inclusion:-

- Containing non-living component such as pigment, food stored (put's Glycogen).

3-cytoplasmic matrix-:

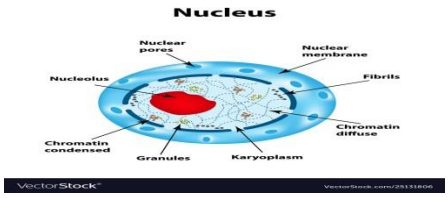
•cytoplasmic inclusions and cytoplasmic organelles are distributed in cytoplasmic matrix

•It contains carbohydrates, proteins, lipids and enzymes. And ions.

Nucleus

- Mostly in center of the call.
- spherical or elongated shaped, containing DNA

* Structure:-



1-Nuclear envelope 2-Nucleolus 3-Nuclear matrix 4- chromatin

1-Nuclear envelope:-

- outer nuclear membrane (with ribosome)
- inner nuclear membrane (No ribosome)
- between outer and inner nuclear membrane
- There is pronuclear cisterna.

2-Nucleolus

Spherical structure, rich in V-RNA and protein

3-Nucker Matrix

Fill space inside nucleus, Composed - stein metabolism and ions.

The types of histochemical specimens

There are 3 types of histochemical specimens:-

1- Vital specimens 2- paraffin preparation 3- frozen preparation

1-vital specimens:-

- specimens are taken from living organs or Living Cells
- This type of specimens allows us to examine the vital cells and tissues.
- The cells and tissues are extracted directly (by using Micromanipulator)" From organism
- The tissues remain alive for 1 to 2 hours and perform their activities.

- This type allows us to do some experiments on the tissue and knowing the effect of drugs on the vital tissue.
- The tissue are extracted from animal and cut it into small. Species and are place on clean (sterile) slides which put on it Points of sterile saline physiological solution, then put the (clean)! sterile cover on the specimens to examine by microscope

*to examine these specimens, we use phase contrast microscope and we can examine those specimens by using light microscope by staining the specimens with suitable stains.

*There are 2 types of vital specimens.

1-unstained vital specimen's 2-stained vital specimens.

A- Stained vital specimens

- In This type of specimens, we use special stain type which are called
- ~vital stains ",ex [Methylene blue, Neutral red stains]
- The stains are dissolved in saline solution and the staining substance. in Solution is more Little and very diluted to keep the tissue alive for Long time [30-45 Min]
- If the tissue remain in stain for longtime, the tissue is poisoned S vital specimens is divided and dead.
- Staining vital specimens is divided into 2 types

1-intra vital staining 2- supra vital staining (Common method)

B-Un stained vital specimens:

-in this type, Vital specimens cannot be examined by light microscope because there are not difference between the cell components and tissue components, so we must use the phase Contrast Microscope For examination.

2-paraffin preparation

• This type is suitable to demonstrate (DNA, ANA) and poly sac hardies and some protein. •This type is unsuitable for demonstrating lipids.

*steps to prepare paraffin specimens

- extract the tissue from animal
- put the tissue in suitable Fixative
- wash the tissue with water
- Put the tissue in ascending chain of alcohols to dehydrate process
- The tissue is put into clearing reagent (Xylene).
- The tissues are placed in paraffin wax and then the tissue embedded in paraffin wax .box.

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3- Frozen specimens

- This type is suitable to demonstrate the Lipids and fats and the sites of enzymes activities.
- In this type, the specimens are rapidly frozen by [Cooling-electrical Cooling] or by using Co_2 gas.
- This means that the specimens are embedded in ice
- Then, we Cut frozen specimens by using [freezing Microtomes or Cryostat].

cryostats

*cryostat [cryo meaning cold, stat meaning stable]

- It is a device for maintaining cold temperature

-It is a microtome but placed in freezing box (cold box)

- It uses in frozen section, and with tissues are treated. In Cold Conditions (-30°c) usually from (-20c:-30 C).

*cryostat technique

-This technique differs from freezing in costume as in this Case, The microtome, The knife, and tissue block are all at the same temperature [-12° to 22° c]

-There is different types of cryostat such as Coon's cryostat Lang cryostat - modern cryostats.

-Temp. May be varied, depending on the tissue being out.

optimum conditions for cryostat…?

-optimum conditions for frozen sectioning

-Water could be considered (regarded) as the embedding medium and sectioning there fare, as a matter of cutting ice.

- The optimum temperature values of the knife, the chamber, and tissue black varied from one tissue to another. However Cutting was usually possible within a wide range of low temp.

*Tissue temperature.

- As in the case with ordinary freezing microtomes, when the temp. Falls below -45°C the tissue will brittle and friable and can't be cut

→Black temp. Between -15° to -4%c it was found possible to Cut thin sections (make cutting Sections easy)

→At higher temp. Up to 60°C, thin serial sections could be obtained.

→Temp. Between -10° to -30°℃, good sections could be obtained.

* Chamber temperature:-

-A chamber temperature between or to -10c, It found that the best conditions for (cutting sections)

*Handling of sections after cutting

-There are number of different techniques and the most widely used.

-Handling of sections is the most accurate processes.

-Sections are taken on warm or cold slide, and then Immerse in cold medium.

Write on "Freezing Microtome"..?

-Freezing Microtome: is high precision scientific instrument that is used for cutting thin to semi-thin sections of fresh I frozen tissue and semi- thin sections.

-freezing Microtome [MION = small, tom to out] * -microtome is an instrument for making thin sections. For Microscopic examinations and freezing Microtome is one of type of microtome for Cutting frozen tissues into thin sections.

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functions of fixation

• Fixation is the main process to prepare the Cell and tissue for staining and Microscopic examination.

*The Three main functions of fixation :

1- Preservation of tissues 2- prevention of diffusion or less f tissue materials 3- Hardening of paraffin specimens.

1- preservation of decomposition

-one of main aims of fixative is the preservation of tissue in Same state of that in the life and at the same time the fixative shouldn't alter the chemical structure or patters of the presence of cell.. And tissue components...

- when tissues are extracted from organism exposed to different changes:-

1-if the tissues are left in air, the tissues will dry and shrink and bacteriological changes and autolysis occur in it (due to Chemical reactions which occur by enzymes which are found in the tissues)

2- If the tissues are placed in water, they will rapidly swell and lose their distinctive features.

- To avoid these changes, we must do the followings:-

1- Fixation of tissues must be rapidly to prevent the autolysis and other processes.

2- Choice the suitable fixative which stops the chemical reactions takes place by enzymes without removing these enzymes.

3- Fixative must not cause swelling or shrinking a tissues and Cells.

2- Prevention of diffusion or loss of tissue material.

- Sometimes fixative may cause change in Localization I the chemical Components of the tissue or loss them outside tissue.

Ex: Glycogen Light phenomenon: In fact the Glycogen is diffused in cell, but in the Fixing tissue. Glycogen is amounting in specific sites of the call and the other sites of the Cell are empty from Glycogen, and thus this not identical to the nature

- To avoid the Glycogen flight phenomenon:-

- We using frozen section or osmic acid.

- in sometimes is required Convert the Cell Components to insoluble Compounds and the staining of them become very easy, 30 the choice of suitable fixative is very important
- To preserve lipids, we use formalin fixative and Fleming without acetic acid.

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3- Hardening of paraffin specimens

- The tissue must be hardened by fixative before catting paraffin section.
- Hardening of tissue allows very soft and friable tissue to be handled without damage during the treatments before the embedding.

- if we left the tissue in fixative for long time, the tissue become over harden and dry, thus the cutting of specimens become hard.

Other functions:-

* protecting the tissue from subsequent treatments:-

- After fixation, the tissue is generally processed (or treated) either through paraffin wax or frozen section to Cut.
- In both cases, the tissue undergoes to fluids or actions (melting) which will Cause loss of substances in the first technique or diffusion I substances in both techniques.

* It can be overcome by.

- By using suitable fixative to preserve tissue components.
- By using formalin fixative before cutting the freezing tissue sections.

✓ Effect of fixative on staining reaction

- Some fixatives alt as mordant
- A constituent (or component) of the Fixative making Chemical linkage between dye and tissue

*The Choice of fixative depends on 3 factors:-

1-The type of stain or reaction which the tissue is to be applied it in future. 2-The size of tissue. 3-whether there is any urgency or not. (Hurry).

*Rate of Fixative penetration

- The rate of penetration varies with different fixatives.
- The rate of penetration depends on tissue types which we want fixed it.
- Before 4 tissue can be considered fixed, the fixative must have fully i Penetrated it.

- The fixative has a fast rate of Penetration is Better than the Fixative has a slow rate of penetration.

*Example

- Formaldehyde has a rapid rate Compared with picnic acid.
- Osmic acid has a slow rate of penetration.

*freezing instead of fixation

- Some histochemical procedures (methods) required to use unfixed. materials (enzymes, hormones, lipids)
- When this is required, the tissue i must be rapidly frozen, directly after removing it from body.

*the freezing of the tissue preserves it by:-

1-stopping the autolytic enzymes from reacting.

2-stopping the chemical reactions.

3-bringing the tissue to a solid state.

formalin fixative [formaldehyde fixative]…

- formally is a solution from dissolving formaldehyde gas in the water [formalin→ Formaldehyde " gas" +dis. H_2o]
- Solutions of formalin are acidic due to the formation of formic acid in it.
- To convert the acidic nature 4 formalin sold to neutral, we add Buffer Soln. or add amount of calcium carbonate, then we filter the Solution.
- The longtime of fixation in formalin, Causes the staining process of tissue (with acidic dye (such as eosin) is very hard.

* Uses & formalin:

1- It is a good fixative for demonstrating the lipids.
2- When adding calcium to formalin becomes a good fixative for preserving (phospholipids).

3- Presenting hydrolytic enzymes (by fixation in formal calcium) at 4°c.
4- Fixing the freezing dried tissue and give a very good results.

*chemical formula

(CH2) Cho . Cho

the chemical action of the formalin in tissue

*The chemical action & formalin

- the reactions of formalin are numerous and complicated, because It reacts with many proteins which are precipitated in the cell + tissue
- some of these reaction are irreversible and other are reversible (reversible reactions occur by washing the tissue with water)

1- Formalin converts the compounds which contain reactive hydrogen (H_2 atom) atom to hydroxyl compound

$RH + CH_2O \leftrightarrow R.\ CH_2\ (OH)$

2-The hydroxyl groups are also reactive group and react with reactive hydrogen atom to form [Methylene bridge] that links the protein molecules.

$R\text{-}CH_2\ (OH) + HR \leftrightarrow R\text{-}CH2\text{-}R + H_2o$

- These methylene bridges are easily ruptured by hydrolysis
- Methylene bridge may be formed between 2 similar groups [(NH_2) (NH2)] or between 2 different groups [(NH2), (NH)] [NH2, CON H].

R-C↓H-C00H CH2O R-C↓H-COOH $R'NH_2$ R = C↓H-CooH

NH2 $HOCH_2$- NH R-HN-CH2-NH

3-Formalin Combine with carbine which is the main protein in the skin and hairs (at pH 6-8) without effecting on the Linkage between (S-S) in Cysteine.

- In more basic medium, the formalin reduces (s-s) to (SH-SH) and form Methylene bridges in same state ($S-CH_2-S$)
- The groups which inter in fixation of the proteins are Peptide groups, hydroxyl, carboxyl group and groups which Contain sculpture.
- We can remove the formalin from tissue by washing the tissue with running water for 24 hours then washing tissue with distilled water.

alcohol Fixative…

- Alcohol is better than acetone for the frozen section for demonstrating the enzyme activities.
- Alcohol and acetone are used for precipitation the proteins. And also used for demonstrating the enzyme activities.
- Alcohol (80% Conc.) used to demonstrate the glycogen.
- The effects of alcohol and acetone are reversible proteins can be returned their main properties when are separated Prom alcohol and acetone.
- It's disadvantages: -

1-precipitate proteins.

2-dissolve (or Remove) lipids.

3-Destruction + Golgi apparatus and mitochondria and secretory granules.

4- Makes the freezing of tissues and the subsequent sections difficult.

acetone…

- Acetone is used in the frozen sections at temp. Between [zero -4°C].
- It is also used in the paraffin sections which demonstrate the Hydrolytic enzymes activities.

- Acetone is more effective as fixative but causes shrinkage of the tissue, so this fixative is uncommon in uses.

aldehydes fixatives [such as Glutaraldehyde

- Glutaraldehyde has the chemical formula [(CH2) CHO-CHO] and structure into the best
- It is the best fixative for the electron microscope"
- It preserves the general structure of cells and tissues without any loss of enzymes and it only occurs in not prolonging the fixation period in this substance.
- It gives a good result to the case of phosphatase and Esterase enzymes specially.

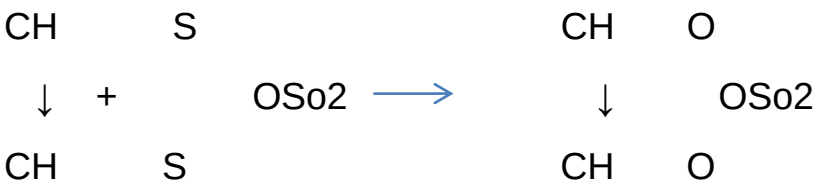
the chemical action of osmium fixative/osmium Fixative…

- It is also called "osmium tetroxide" Cosmic acid) "OS04 ".
- It is anti-Coagulant fixatives.
- It is a good fixative for demonstration of lipids, and also used in the electronic Microscope preparation.
- It preserves (or maintains) the fats in insoluble state and is used (with Conc. 1%) in buffer solution
- Preserve the mitochondria and Golgi apparatus but it has a slower rate of penetration of the tissue and cell.
- If the tissue are left in it for long time, the tissue becomes friable and dry, over harden.
- If the tissues are left in it for short time, the internal parts of tissue. Remain UN fixed, so we must know the suitable time of each type of tissue which the tissues are spent in fixative to fully fixed.
- It is poisoned substance and cause harmful effects for sight (vision) and smell (sniffing).

* Influence of osmic acid

- unsaturated fats reduce the osmic acid and produce "black Compounds which may be contain "osmium element or

osmium hydroxide, this is occur because of the oxidation of double bonds between the adjacent carbon atom.

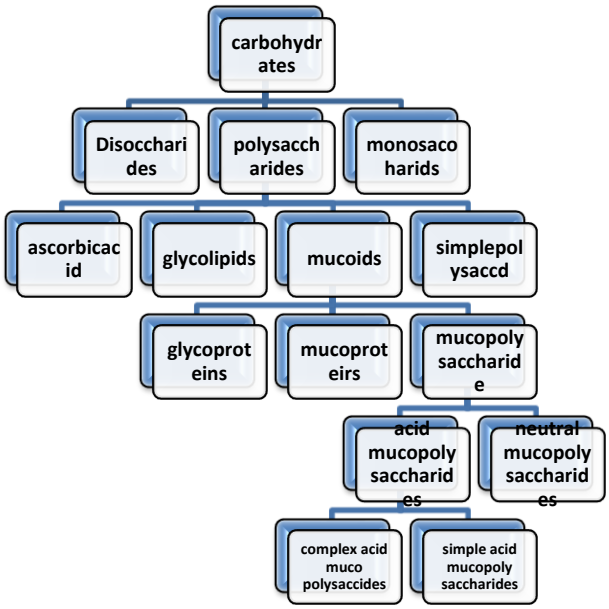


*the osmic acid solution (2%) with albumin and fibrin gen form a gelatinous substance.

picric acid …

- It is one of the most important fixatives and most widespread.
- It fixes the samples well without causing shrinkage in it
- It is used in many compound fixatives, especially for demonstrating Glycogen.
- Tissue which fixed in picnic acid, it must be washed well by alcohol or water until the yellow color is removed from it.
- It precipitates proteins and combines with some of them.

classification of carbohydrates.



*carbohydrates

- Organic Compounds are formed of [C, H, O] and these compound! are formed inside plant-tissues from Co2 H2O through photosynthesis

$Co2 + H2O \rightarrow C6H12O6$

- Animals obtain the Carbohydrates through Feeding on plants where carbohydrates are digested and transformed into simple substances can be easily absorbed.
- These substances are the most important source for energy.
- They are considered as the main component for many bios chemical Substances.

1- Ribose → Nucleic acid 2- GA lactose → Fats

3- Lactose → Milk 4-neutral macro poly saccharide → chitin

5- complex acid muco polysaccharides → Heparin

*chemically, carbohydrates are defined as aldehyde or ketones.

mono saccharides + disaccharides…

- Mono saccharides and disaccharides are known as Sugars because their sweet taste and both soluble in water, when they dissolved in water form transparent solutions which can pass through cell membrane

1- Mano Saccharides (Co H206)

- They are simplest Carbohydrates and they can't be hydro zed into simple sugar
- General Structure : [Cn (H2O)n] hexoses
- They may be classified into trioses tetroses , pentoses , depend on the number of Carbon atoms
- They may be classified into ketones and Aldoses depend on the type of group which contain it
- pentose and Hexoses are more distributed in the Cells and tissues of the animal and may be combined with proteins or lipids
- pentoses are one of components of melic acids

-There are 2 types of pentoses: _

1-ribose is one of main components of RNA.

2-Deoxyribose is one of main component of DNA

3- Hexoses are represented in Glucose + GA lactose + fructose.

2-Disccharides (C12 H22 O11)

- It is formed as a result of combination of & molecules mono saccharides and Loss water molecules.
  2 C6 H12 O6 → H2O C12 H12 O11
- when the Disaccharides are digested or hydrolyzed, give 2 molecules of mono saccharides and water molecule and this process is "reversible process"

2 C6 H12 O6 → H2O C12 H12 O11
← +H2O

*example

- Disaccharides such as [Maltose, sucrose, lactose ,Cello bios.

* Polysaccharides (C6H12 O5) n

- They are formed by condensation (or combination) of many. molecules of mono saccharides with loss water molecules

N C6 H12 06 → n H2o (C6H10 O5)n + H2O

- They are not soluble in water or alcohols, and forming Colloidal solution and don't plass through cell membranes.
- The most important of the polysaccharides are starch and, Cellulose in plants and glycogen in animals.

starch, Inulin, Cellulose, Dextrin

- Starch

- It is the stored (reserve) substance in plant cells

CO2 + H2o → photosynthesis starch

- Starch is hydrolyzed to give (glucosan) and Neutral starch gives a blue Color with Iodine soln.

- inulin

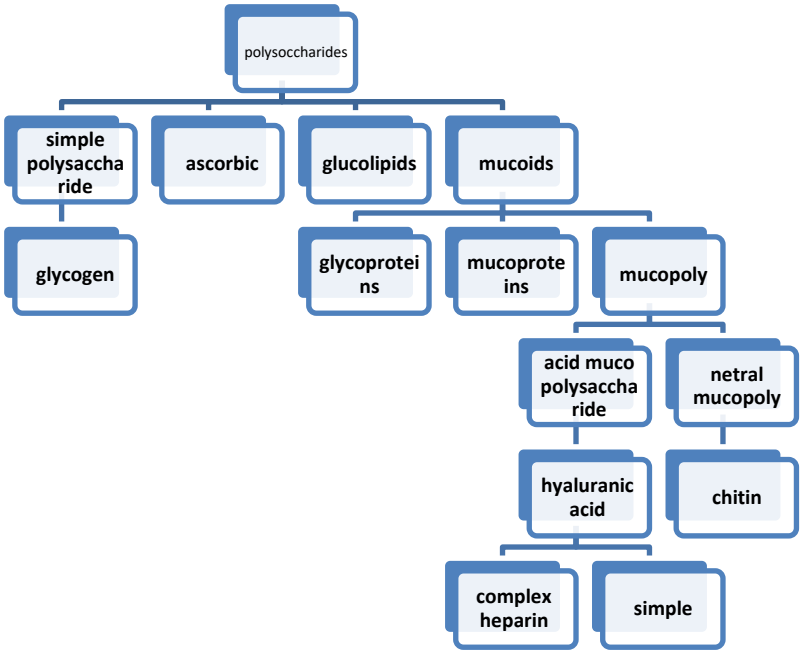
- It is type of starch and stored in roots and tubers of some plants.
- It hydrolyzed into fructose and don't give any color with iodine soln.
- It used to determine the glomerular fit vate (GFR).

- Dextrin

- They are substance which are formed by hydrolysis of starch (digestion -f starch)
- It gives a red color with iodine [so, they called "erythrodextrins "].

- Cellulose

- It is the main component of most plant cell walls.
- it formed from Glycosides
- It is not soluble in water
- It doesn't give any color with Iodine soln.



Glycogen and it's flight phenomena…

- Glycogen is the simple polysaccharide and it is formed" from No of Glucose molecules.
- It is the reserved substance in the animal cells and tissue So it is known as "animal starch"
- The extraction of starch from plant call and tissue easily but the extraction of Glycogen is more difficult
- It present in liver cells and muscles cells and in other cells. With little amount.
- It is found in forms of small granules combined with proteins So, any fixative is suitable for protein and is also suitable for glycogen
- It is little soluble in water (10-15%)

* Shape of Glycogen

*Glycogen can be classified into :-

1- Liver glycogen (which found in Liver)

2-muscle glycogen (which found in muscle cells)

*There are 2 types of glycogen in liver cells:_

1- lyo glycogen: it is amount of glycogen which are hydrolyzed rapidly and are lossed from tissue and cells (rapidly hydrolysis)
2- Desmo glycogen, it is an amount of glycogen which remains for period.

*Source of glycogen:_

1- Mono saccharides which are result of digestion + sugar and Starch (carbohydrates in the alimentary Canal.

2-Lactic acid; which is generated in muscle cell.

* Glycogen flight phenomenon.

1-in Living Liver cells

- The glycogen is diffused in cytoplasm of all (not found in nuclei)

2-in fixing liver cells

- Glycogen is not diffused but is accumulating in specific sites! Cell (fake crescent-shape) and the other sites of cell are empty from glycogen and this not identical to the nature state.

*To avoid the Glycogen flight phenomenon.

1-use (drying frozen sections)

2-use osmic acid (in fixation), where the tissue is exposed to diluted Solution of osmic acid for short time before fixing. It by ordinary fixatives.

Mucoid substances

- It is carbohydrate substances, formed from monosaccharide molecules but contains " amino group (-NH_2) instead of hydroxyl group in sugar (Glucose), so it called " Glucosamine" and these substances play an important role in absorbing water + activating Bowel (intestine) movement.

*classification

1- Mucoproteins

- It formed from Combination of Glucosamine with proteins Dipeptides

* تتكون من اتحاد وحدات سكرية امينية مع بروتينات ثنائية النترات وتشكل السكريات الامنية 4% من هذه المواد.

- They are positive PAS or give positive reactions with shift regent.

* تعطي تفاعلات ايجابية مع محلول شف وايضا تصبغ (Bromophenol blue) الخاص بتميز البروتينات.
* توجد بكثرة في افراز الغدد اللعابية + الهرمونات جنسية.

2- Glycoprotein

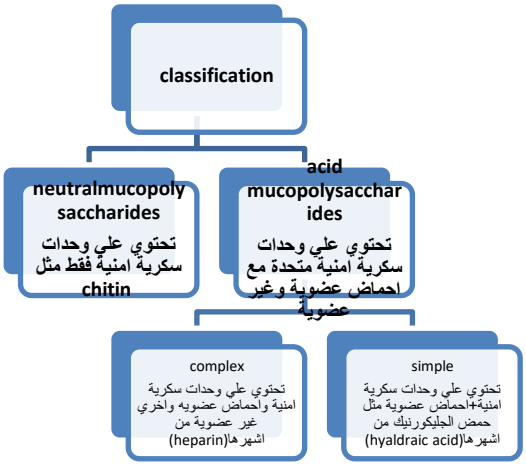
* مثل (muco proteins) ولكن نسبة السكريات الامنية تكون اقل (اي تكون اقل من 4%).

* توجد بكثرة في مصل الدم + بياض البيض.

* It contains less than 4 % of glyco5amine.

Muco poly saccharides

* تتكون من وحدات سكرية امنية فقط او متحدة مع بعض الاحماض العضوية مثل حامض الجليكورونيك او غير عضوية مثل حمض الكبريتيك المركز.



chitin, Hyaluronic acid Heparin…

1- Chitin

- It is the simplest neutral muco polysaccharides.
- it consists of building units "glucosamine"
- Chitin is demonstrated by Schiff reaction and gives reddish purple color.
- It is found in the exoskeleton of the insects and other arthropods, and in Mollusca, insect larvae, in fungi

- It is the most in soluble organic substances, but it is soluble in not HCl, H_2SO_4.
- Chitin similar to Cellulose, in both are in soluble in water but Cellulose soluble in "Cupric ammonium hydroxide bat chitin in soluble in this substance.

2- Hyaluronic acid

- It is simple acid muco polysaccharides.
- It is composed of amine sugar units + glucuronic acid.
- This acid is existing in highly polymerization form" It forms skin shell and eggs shell. [skin + ova coat]
- It is soluble by Hyaluronidase enzyme.
- It is known as glycocalyx.

3- heparin

- It is complex acid muco polysaccharides.
- It is composed of [amino sugar+ glucuronic acid + sulphuric (or phosphoric) acid]
- It presents mainly in mast cells.
- It is secreted by liver calls, and it is an anticoagulant substance.

*other poly saccharides…

3- Glycolipids

- it composed of sugars + lipids
- -It found richly in neurons such as Kerasin, Phenosin
  Glycolipids → hydrolysis amino acid + sugar+ sphingosine.
- -It gives a positive reaction with Schiff reagent (positive PAS). and a stained by lipid stains

4-Ascorbic acid or Vitamin.

- It is a derivative at carbohydrates.
- It is act as Coenzyme in redox reactions
- -It found richly in plant tissues but found rarely in animal tissues and exist in (adrenal cortex)
  Silver nitrate + vitamin C→ dark granules

PAS Technique…

•periodic acid-shift reaction (DAS)

-This method is the best histochemical method to demonstrate the carbohydrates.

- This methods involves 2 processes [oxidation + then staining]

1-oxidation process

-in this method, the periodic acid (HI04) is used.

-The oxidation takes place by breaking the linkages between (C_2-Cs) atoms which present in glycol group Perms (112) convert the glycol groups into aldehyde groups (HCO-HCO)

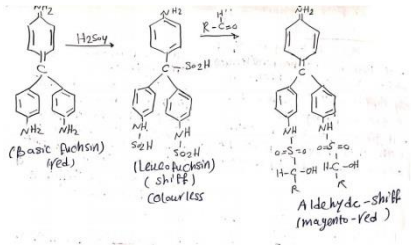
-periodic acid (HI04) are not oxidized the aldehyde groups which are produced >se carbohydrates (contain aldehyde groups) are ready to react with stiff reagent and give a heavy purple Color.

2-pentration of Schiff reagent.

- The producing aldehyde groups combine with "Leucofushin" (Colorless) which is result from Schiff reagent and producing "Dark purple Color Compounds"

- The amount of Color depends on the amount of reactive glycol groups in tissues.

-PAS reaction also can be used to demonstrate General Carbohydrates and muco protons and neutral muco poly saccharide and glycoproteins [giving dark purple color]



*طرق اثبات صباغة المواد الكربوهيدراتية:-

A)طرق انزيمية:-

1- انزيم (amylase) ← glycogen

2- انزيم (diastase)

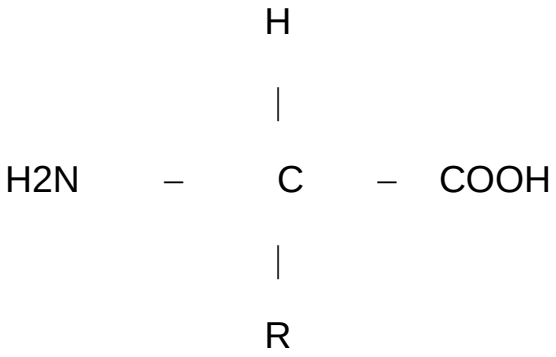
3- انزيم (Hyaluronidase) ← (hyaluronic acid)

B) طرق التوقف او المحاصرة:-

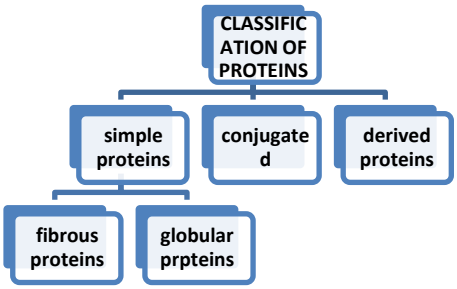
[Acetyl anhydride → توقف effective of glycol]

Proteins

- Proteins are the tissue builder (or structural substances) because they are found in all and tissue of the body, and their bull Unit "amino acid". And elements "o, HICIN" and other elements may enter in their structure "p, s, f e, I.
- Proteins are Organic Substances of high molecular weight. And they are formed by a number of amino acids...
- Amino acids are organic acids in which the α Carbon atom that addacent to carboxylic group link with amino group.
- Amino acids are called "amphoteric" because Contain (an acid group + basic group].



- Amino acids could be obtained from proteins by hydrolysis under the effect of enzymes or boiling with acid or bases.
- Amino group (-NH_2) of one amino acid + carbonyl group of another amino acid Fermoy dipeptide.
- Large number of amino acids united together form Poly Peptide.



Proteins

1- simple proteins:-

They are formed by hydrolysis amino acid

A) Fibrous proteins:

- In which the polypeptide Chains are arranged in α-helix or B-helix in Plat form
- Insoluble in water (Collagen Keratin + myosin + Elastic fibers + fibrinogen + fibrin + Reticulum).
- Soluble in aqueous sol (myosin + fibrinogen).

B) Globular proteins:

- They have quadrant Or triplex structure and form "globular shapes"
- All soluble in water/aqueous
- Albumin, protamine + Globin + Globulins + Histones.
- Globular proteins may convert into fibrous proteins. By exposed it to certain temp. And specific PH.

2- conjugated

- They are formed by hydrolysis other subs. of non-protein nature
- They are complex proteins and contain one or more molecules from other substances which is called "prosthetic group" (phosphor – lipo – glycol - muco)

Ex:

- muco proteins + Glycoproteins
- lecithin proteins
- Chromo proteins

3- Derived proteins

They include compounds + Coagulated Proteins + Partially soluble proteins soluble proteins.

Collagen, elastic fibers, Histones….

1- Collagen:-

- It is the most common fibrous protein in human body
- it composed of amino acids [glycine + proline + hydroxyproline + hydroxyl Lysine]
- It is secreted by fibroblasts as procollagen.
- Collagen fibers are flexible but inelastic.
- Collagen is acidic dye (acid ophitic stain) and gives pink color with eosin stain.
- Collagen forms gelatinous substances in boiling water.
- There are 3 forms of collagen according to their solubility

A. Acid Soluble Collagen; is extracted with dilute acetic acid.
B. alkali or neutral soluble Collagen: It can be extracted from skin with a weak alkaline phosphate buffer (or means of neutral salts)
C. Insoluble collagen which causes the bulk of tissue collagen.

2- Elastic fibers / tissue:-

- It is a fibrous protein and it is known as "elastic fibers.
- Elastic fibers are less thickness than collagen fibers (more thin)
- Elastic fibers have yellow color I so they called "yellow fibers elastin is formed by fibroblasts and found in [arterial walls + alveoli walls + connective tissue].

- Elastin composed of large no. of amino acids more than Collagen [such as, valine, Alanine, Desmosine, isoDesmosine…]
- Elastin contains more lipids than collagen.
- It is not affected by boiling and acids or bases.
- It is not digested by trypsin but slowly digested by pepsin and easily digested by Elastase.

3- Histones:-

- They are simple Globular proteins". and are known as basic Proteins" because they are composed of large no. of basic amino acids (histidine ,arginine, lysine)
- Some of Histones combine with nucleic acid to form (nucleohistone)
- They are found in nucleus of Eukaryotic cells in 4 Forms [H_2A, H2B, H4 1H3] but not found in prokaryotic cells.
- Histones are soluble in water I diluted acid or base soln. but insoluble in diluted ammonia solutions.
- It is found in pancreas and thymus gland.

keratin Reticulum, protamine…

1- Reticulin

- Reticulin is fibrous protein, consists of fin branching fibers and known as " Reticular fibers"
- Reticular fibers are thought that are incomplete collagen fibers but Reticular fibers consisting of collagen substance III, but the Collagen Flores consisting of collagen subs. I.
- Reticular fibers give a positive PAS reaction and are not give gelatinous Subs. by boiling.
- Reticulin contains meristic acid.
- Reticular fibers are not seen with eosin or hematoxylin but are seen in dyes which are treated by sliver salts, so... reticular fibers known as "Argyrophilic fibers."

2- Keratin

- Keratin is simple fibrous protein and mainly found in the epidermal all of birds, mammals reptiles, and also in skin derivatives (such as hair, feather…).
- Keratin Composed at basic amino acids [arginine, Lysine histidine] and rich in Sulfur and high ratio of cysteine.
- Keratin provides Large chemical and mechanical a protection to tissue which is below it.
- It is insoluble in water and diluted acids or bases and has strong affinity for basic and acidic dyes and is not digested by pep sine and trypsin.

3- Protamine

- They are simple Globular proteins and basic proteins.
- Protamine also combine with nucleic acids (like Histones) and they are represent the main component A chromosomes
- They are soluble in water, and diluted acids.
- They are discovered for first time in spermatozoa of fishes.

Identification of proteins

millon's reaction…

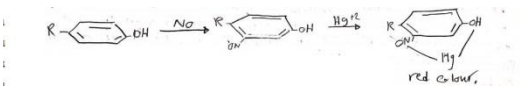
This reaction is used to demonstrate the proteins which have Phenolic group.

This reaction occurs in 2 stages:-

1-nitrophenole is produce by the substation for H crouton hydroxyl of the phenol.

2- Hg+ enters anew ring that contain nitrogen of Nit rose group

*the new complex is" red color"



-Millon's reagent formed by digesting Mercury nitrate in nitric acid and dilution the result so in with water.

Other methods

1-mercury – Bromophenol blue

-The stain is prepared from mercury chloride and Bromophenol blue and the proteins are stained with heavy blue color.

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2-Acrolein-shift methods

-The method stains the proteins generally

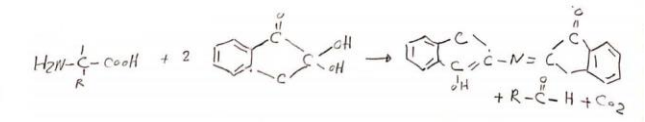
- We processing the section with Achroline (H_2C=CHCHO).

-the double bond of acroline react with the NH_2, SH, NH and Imidazole and leave the free aldehydes to react with Schiff reagent and produce purple red color.

3-Ninhydrine Schiff methods

-This method is used to demonstrate proteins which contains reactive amino group,

- The sections are processed with ninhydrin and form compound has "blue color" and Co2 Compound contains aldehyde group and sections are washed by water and are placed in Schiff reagent that reacts with aldehyde Compound to produce "purple red Color" .



General structure of Nucleic acids …

- Nucleic acids are Large molecules (polynucleotides), are 2 types deoxyribonucleic acid (ANA) and ribonucleic acid (RNA, are found in animal and plant cells, and are usually combine with basic protein. to form nucleoproteins.
- DNA is found in nucleus, and represents the genetic materials in which genetic information are stored.
- RNA is found in cytoplasm and a small amount of it occurs in nucleus and it controls protein synthesis."

Hormones

- DNA → RNA → Protein

Enzymes

* The structure of Nucleic acid protein

-Nucleic acid generally composed of :-

1-Nucleotides. 2- Phosphate group. 3- Pentose (or five carbon) sugar 4-Nitrogenous bases [purines + Pyrimidine's]

* Nucleotides are the building unit of Nucleic acids, and each nucleotide Composed of

1-Five carbon sugar. 2-phosphate group. 3-Nitrogenous bases.

1-five carbon sugar (pentose sugar)

- It linked to phosphate group [at five carbon atom]; and linked to nitrogenous bases [at 1 carbon atom].
- In DNA, pentose sugar is deoxyribose, but in RNA, pentose sugar is ribose.

2-Nitrogenous bases

- They are purines and pyrimidine

A) Purines are dicylic compounds that include [Adenine [A] + Guanine (G)]

B) Pyrimidine are Monocyclic compounds that include [cytosine (C) + Thymine (T) or uracil (U)].

- In DNA, purines bases [A,G] and pyrimidine (c, T) but in RNA ,Purines [A, G] and pyrimidine [C/U].

*Adenine (A) Links with Thymine (T) or uracil (u) and Form together & hydrogen bonds"

*cytosine (C) Links with Guanine (G) and form together 2 hydrogen bonds

*nucleotides are linked by phosphodiester bond"

- if a phosphate group is removed from the nucleotide, the resulting compound is called" nucleoside

[Adenosine, guano sine, thymidine, uridine, cytidine].

the General Reactions for nucleic acid…

1- Reaction for organic phosphate:-

- The phosphate, radical is demonstrated in DNA of the Nucleus by hydrolysis and subsequent fixation of (Po4) groups which are treated with ammonium moly date.
- The resulting phosphor moly date is reduced to blue compounds with benzidine.
- It uses enzyme hydrolysis with nucleases or prolonged hydrolysis with N-HCl.
- This method is used to demonstrate the presence of (Po4) in the chromosome .

2-Reactions for purines and pyrimidine

- The tetrazonium reaction after benzoylation or acetylation is only reaction which may be used for demonstration of the purines and pyrimidine .

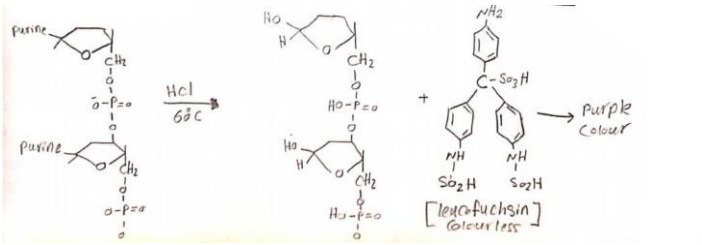
3-Reaction for Deoxyribose and ribose

- This method depends on mild acid hydrolysis which followed by reaction. With phenyl (or methyl) trihydroxy fluorine.
- DNA is stained with violet to blue black color.
- RNA is stained with yellow to red color.

the feulgen [Feulgen- Schiff] reaction

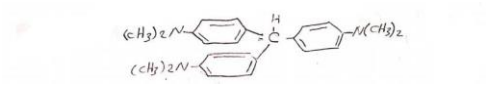
This reaction depends on the treatment of fixed tissue by Mild acid hydrolysis (with N-HCl at 60°C) which could remove the purines and releases (liberates) the aldehyde groups from the deoxyribose sugar (of DNA) only.

- Then, the tissue are washed and transferred to Schiff's reagent which react with aldehyde groups and produce "purple color"
- This reaction may be depend on the ability AN-HCl to break down the linkage between C, and C_2 in Deoxyribose only and not on ribose, therefore, the aldehydes groups form only in only DNH not RNA.



Methyl Green -pyronin method for DNA, RNA…

- This method is used for demonstrating of DNA and RNA.
- (Methyl green) is an impure dye which contains methyl violet, thus, it must be washed with chloroform to remove Methyl violet.
- It a basic dye, so it has an affinity for DNA [DNA has acidic properties].
- Dye combine with DNA molecules in 2 sites [2 amino group on the dye Combine with 2 phosphoric acid group of DNA
- Pyronin is impure dye, and it must be washed with chloroform to extract the impurities.
- It has an affinity for RNA the results: DNA is stained with green color, RNA is stained with red color.



*other examination

- Methyl green has affinity for DNA and Pyronin has affinity d or RNA
- When, we use 2 specimes, one of them is treated with rib nuclease. And in (this section), The DNA is stained only and this is examined by the Pyronin stain, the nucleic acid that has less degree of Polymeration.
- The DNA when it becomes less polymerization can be stained. With Pyronin like RNA.

types of RNA…?

- There are 3 types of RNA : [M-RNA , Y-RNA T-RNA]

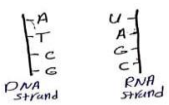
1- Messenger RNA (M-RNA):

- It is transcripted from a specific part of one strand of DNA
- The length of M-RNA is depending on the length part of DNA which mRNA is transcripted from it.
- The sequence of Nitrogenous bases of M-RNA depends on the sequence of nitrogenous buses of DNA strand. (complementary base pairing)
- M-RNA represents the Code for protein [sequences of bases determine the amino acids arrangement].

DNA transcription RNA translation protein

2- Ribosomal RNA (r-RNA):-

- The ribosome consists of 2 subunits one Large and the other is small, each subunit composed of one molecule of r-RNA.
- R- RNA is representing the place of m-RNA with t-RNA.



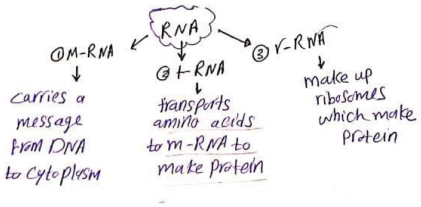
3- Transfer RNA (+-RNA):-

It consists of Long strand + RNA which is coiled around itself to form (clover-shaped).

It contains anticodon which is formed from the sequence of 3 nucleotides and it's free end Linked to a specific amino acid.

There are 22 types of t-RNA .

It is moved along the m-RNA until it's anticodon in it found the Codon in mRNA strand and the position of amino acid is determined.



RNA types and proteins synthesis / protein synthesis as example to prove the cell is structural and functional unit in all organisms…

A) Types of RNA:

1- M-RNA: It is transcripted from special part of one DNA strand .

- It carry message [instructions] from DNA [in nucleus] to Ribosome [in cytoplasm] for protein synthesis.

2- r-RNA:

- It makes up ribosomes which consist of 2 subunits one is large, other is small, each of Ribosomal subunits is composed of one molecule of r-RNA It represents the place of M-RNA with t- RNA

3- t-RNA:

- It contains anticodon [for codon in m-RNA] which consists of 3 nucleotides and the free end linked to special amino acid.
- It carries amino acids to Ribosome for protein synthesis.

B) Protein synthesis:

- It is the process in which cells make proteins based on message [instructions] From DNA which carried by m-RNA .
- It includes 2 stages, transcription [in nucleus] and 2 translation [in cytoplasm].

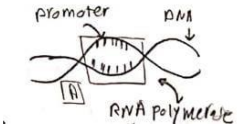
1- transcription (DNA RNA) :-[in nucleus]

- It is the process in which genetic information from DNA strand is copied into m-RNA strand.
- This process is like DNA replication, but in this case, DNA acts as a template for building m-RNA not new DNA.
- It occurs in 3 steps:

a- Initiation . b- Elongation. c- Termination.

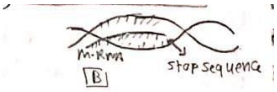
a- initiation [the beginning of transcription] :

- Like DNA polymerase, there is RNA polymerase starts to bind to a specific region on DNA strand called "promoter"
- RNA polymerase also works on 5 to 3' direction.



b- Elongation

- As RNA polymerase moves along DNA, It untwists [separates] the double helix, then adding bases by base pairing rules. To form "M-RNA".



c- termination [the ending + transcription] [M-RNA is complete and leaves nucleus]

- Transcription process continues until RNA polymerase transcribes terminator (or stop) sequence .

- Once m-RNA strand is complete, it releases from RNR Polymerase and leaves nucleus and then sent to cytoplasm.

2-translation[RNA →protein] : [in cytoplasm]

- It is the process in which the genetic code in m-RNA is read to make protein

[Genetic code in m-RNA → chain of amino acids(Polypeptide chain) → Protein].

- It occurs at ribosome (in cytoplasm) in 3 stages:

1- Initiation. 2- Elongation. 3- Termination.

a- initiation [beginning of translation]

1- Each ribosome has a binding site for m-RNA [contains codons] and 3 binding sites for t-RNA [contains anticodon + carries amino acid].

- The 3 binding sites of t-RNA:-
- These 3 binding sites found in large ribosomal subunit of ribosome.

1- P-site in which + RNA with First amino acid binds to it.

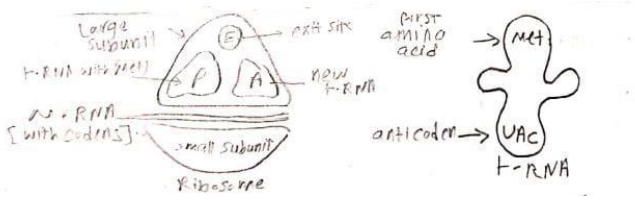
2- A-site in which new +-RNA with Next amino acid binds to it.

- A peptide bond forms to Link the amino acid of t-RNA in (P-site) with amino acid of t-RNA in (A-site).

3- E-site [exit site ++RNA]: F-RNA leaves Ribosome (without It's amino acids] at e-site.

2- This process starts when ribosome binds to m-RNA and begins Looking for start Codon" (AUG) in m-RNA.

- When it is found 2 ribosomal subunits on it together to form. Functional ribosome, then RNA which carries methionine (met) [first amino acid in every protein] enters P-site, and translation Process begins.



b- Elongation

1- T-RNA which carries methionine (met) [first amino acid] enters (P-site) in large ribosomal subunit.

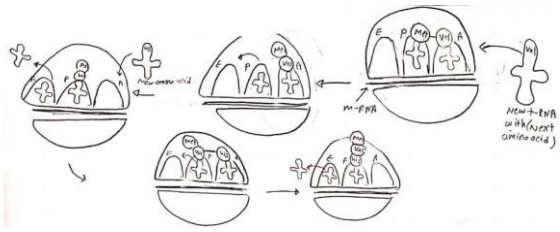
2- T-RNA which carries next amino acid [like vat] enters [A-site].

* The peptide band forms to link [met][or first amino acid] of t-RNA in (P-site) with (vat) [second or Next amino acid] of t-RNA in (A-site).

3- t-RNA leaves p-site and moved to E-site (without it's amino acid) and leaves ribosome, also t-RNA in (A-site) moved to

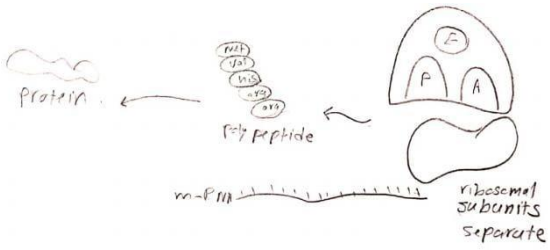
1P-site) I there fare (A-site) becomes empty and ready to receive new + RNA with Next amino acid.

- These steps of elongation process continue to add (amino acids) until the poly peptide chain is complete.



3- Termination[the ending of translation]

- Elongation process continues until one of 3 stop codons [like UAA] is reached in A-site.
- there is no anticodon (in t-RNA) for stop codon, but release factor" binds to stop codon", and hydrolyzes the bond between "Polypeptide clean" and it's t-RNA in P-site.
- This leads to release "Polypeptide chain from ribosome, then polypeptide chain undergoes several modifications and converted into functional protein".



DNA Forms….

* During Forms the cell cycle, the genetic material (DNA) takes different forms

1- In the nucleus, it is found Coiled with Histones to form chromatin.
2- Chromosomes, it consist of one or more of DNA molecule:-

a- chromosome may be consisting of one molecule of DNA and is called thread" single threaded Chromosome" or "single chromatin thread".

b- chromosome also may be consist of 2 molecules + DNA and is called double threaded chromosome or double Chromatin strand or "Thread,"

3- In other phases from cell cycle. It is found in the form of condensed grains (or Chromatin granules).

- this grains may be consist of single chromosome coiled around itself in G_1-phase or double chromosome in G2-phase

DNA replication…

- DNA replication:- is the duplication process & DNA molecule in each S-chromosome to produce d-chromosome, where it' divides in m-phase into 2 s-chromosome thus each daughter Cell contains a chromosome is identical to the chromosome of Parent cell
- Replication process occurs in s-phase under the influence of many important and essential enzymes.

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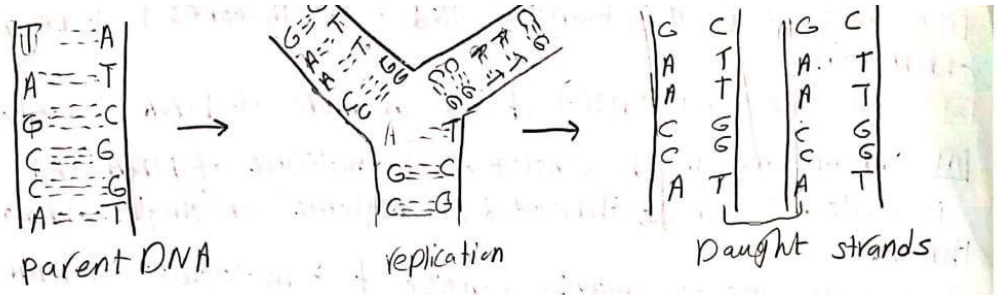
Mechanism

1- The 2 strand of DNA molecules begin to remove their coiling around each other, this begins in specific region of the DNA molecule and then continues until the 2 strands become completely separated under the influence of certain enzymes.

2- Each strand begins (with help of other enzymes) to form Completely Complementary copy of itself. where adenine comes against thymine and vice versa, also Guanine comes against cytosine and vice versa, then every 2 adjacent buses attached together by hydrogen bonds where the bases of old strand a hacked with the bases of New Car Complementary) strand.

3- So, 2 molecules of DNA are formed and each DNA molecule Consists of 2 uncoiled strands one is original and the other is complementary to it, then these strands became coiled around Each other (with help of enzymes) to form one DNA molecule.



*تناسق/ تضاعف DNA :

- هي عمليه مضاعفة جزئ DNA في كل (S-chromosome) لينتج (d-chromosome) حيث ينقسم في مرحلة (M-phase) الي 2(S-chromosome) وبذلك تحتوي كل خلية بنوية علي كروموسوم الخلية الاصلية وتحدث هذه العملية في مرحلة S phase تحت تأثير العديد من الانزيمات الهامة والضرورية.

*ميكانيكية:

1- يبد خيطي او شريطي DNA في ازالة التفافها حول بعضها البعض ويبدأ ذلك في منطقة معينة في الجزيء ولكنه يستمر حتي يصبح الشريطان منفصلان تماما تحت تأثير انزيمات اخري(معينة).
2- يبدأ كل شريط بمساعدة انزيمات اخري في تخليقه نسخة مكملة له تماما بنفس نظام التكامل المعروف ، حيث يأتي كل اوتين مقابل سيمين والعكس بالعكس وكما يأتي جوأتين مقابل اليتوسين والعكس بالعكس ثم ترتبط كل قاعدتين متجاورتين مع بعضها بالروابط الهيدروجينية حيث ترتبط القواعد في الشريط القديم بالقواعد في الشريط المكمل(الجديد) وبذلك يتكون 2 جزئ DNA ، وكل جزئ يتكون من شريطين غير ملتفان احداهما اصلي والاخر مكمل له ثم يلتفان حول بعضهما بمساعدة الانزيمات ويكونا جزئ DNA لولبي حلزوني وفي نهاية يتكون 2 جزئ DNA لولب حلزوني.

Carbohydrates

CARBOHYDRATES:-

- The carbohydrates are organic compound formed of C,H and O .they are widely distributed in both animal and plant tissue.
- In the animal cello, the most important carbohydrates are glucose, GA lactose and glycogen.
- Carbohydrates taken into the body are transformed into simple sugars before being absorbed.
- These sugar are oxidized in the tissue and are the most important source of energy which can be cased for body functions.
- Some carbohydrates have highly specific function in ribose processes, e.x: ribose in the nucleoprotein of the cello GA lactose in b certain fats,
- Chemically, carbohydrates are defined as aldehyde or ketone derivatives of the higher polyhydric alcohol. Also, they are defined as aldehyde or ketone derivatives of compounds which yield these derivatives on hydrolysis.

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CLASSIFICATION OF CARBOHYRATES:-

- Carbohydrates are classified as monosaccharaides, polysaccharides and disaccharides.
- Monosaccharaides and disaccharides are known as sugars because of their sweet taste .they are soluble in water ,and

alcohol . They easily pass through semipermeable membranes .

- The poly saccharides , on the contrary : from colloidal solution with water , do not crystallize and do not pass across living membranes .
- the most important of monosaccharaides are in the cells are pentose and hexoses
- Pentose are one of the main components of nuclear chromatin : pentose ; ribose ($C_5H_{10}O_5$) and deoxyribose ($C_5H_{10}O_4$) intervene in the constitution of nucleic acids .
- The hexoses are represented by glucose (C6H12O6) which is involved in the energetic changes of the cell .
- Other example of hexoses as GA lactose and fructose .

- THE DISACCHARIDES :-
  - They are the result of condensation of two molecules

- THE MONOSACCHARIDES :-

- They are simple sugars which cannot be hydrolyzed into a simple form the general formula is c n (H2o) n .
- the simple sugars may be subdivided as pentose , hexoses (Heptodes) depending upon the number of carbon atoms they possess ; and as aldoses or ketoses depending upon the aldehyde or ketone groups are present .
- the most important of monosaccharaides are in the cells are pentose and hexoses these are frond usually combined will proteins and lipids
- Pentose are one of the main components of nuclear chromatin : pentose ; ribose's ($C_5H_{10}O_5$) intervene in the constitution of nucleic acids .
- The hexoses are represented by glucose ($C_6H_{12}O_6$) which is involved in the energetic changes of the cell .
- Other example of hexoses as GA lactose and fructose .

- THE DISACCHARIDES :-

- They are the result of condensation of two molecules of monosaccharaides e the loss of one molecule of water

$2C_6H_{12}O_6 \xrightarrow{-H_2O} C_{12}H_{22}O_{11}$

- THE POLYSACCHARIDES :-
  - They are formed by the condensation of many molecules of monosaccharaides with a corresponding loss of water molecules :

$NC_6H_{12}O_6$ $\xrightarrow{-NH_2O}$ $(C_6H_{10}O_5)\ N$

  - The most important of the polysaccharide are starch and cellulose in plants and glycogen in animals .

STARCH :-

- It is the reserve substance in plant cell , and sync –
- The sided form CO_2 and H_2O by means of chlorophyll .

$H_2O + CO_2$ $\xrightarrow{\text{CHLOROPLIYLL}}$ $(C_6H_{10}O_5)N$

PHOTOTHENSIS

- By hydrolysis , starch yielding only glucose that it called as glucosan .
- Starch (glucosan) hydrolysis glucose .
- neutral starch give a blue color with iodine solution inulin.
- It is a starch which is hydrolysable to fructose units; it is properly referred to as a fructosan.
- It soluble in warm water do not give color with iodine also it is used in physiologic investigation for the deter mention of the rate of glomerular filtration .

CELLULOSE :-

- It is the main constituent of most plant cell walls and enter in the formation of a series of structures which form part of supporting skeleton of plants . It gives no color with iodine and not soluble in ordinary solvents .
- The polysaccharides found in different forms in the body cells and their chemical nature and physical role differ widely in the different cells .
- They resemble each other in containing sugar and they are identified his to chemically by the reactions of this sugar moiety.

CLASSIFICATION OF POLYSACCHARIDES

THESE ARE CLASSIFIED INTO :-

1- Simple polysaccharides .
2- Mucoid substances .
3- Glycolipids .
4- Ascorbic acid .

- Simple polysaccharides .

- Glycogen is the simple polysaccharides of the animal body , and is called animal starch : it is a branched structure with straight chain units .
- Glycogen is an important reserve of the energy of the body ,and found in-
  Numerous tissues and organs . Specially in the liver cells and muscle fibers.
- Glycogen soluble in water and may be dissolved in the proto Plasma , thus it is difficult to demonstrate it in the living cell .
- Glycogen can be precipitated with various fixative and can be demonstrated histochemical by :-
  Iodine reaction with gives a reddish brown color e glycol.. *
- Glycogen gives a deep violet color e PAS .*
- Glycogen gives a dark red color e best carmine staining *
- To test the presence on glycogen , a control is used before staining the material
- Subjects to the action of diastase or salivary amylase . in this case , a negative result is obtained

GLYCOGEN FLIGHT :-

- Sometimes , glycogen is not uniformly distributed in the cytoplasm of fixed cells particularly in lives cells where it appears accumulated in certain regions of the cells.
- Whereas other regions remain almost empty . This occurs because of the fixatives which are used for preservation of glycogen sweep this material in front of them until it

becomes pressed against the part of cell membrane opposite to the direction of diffusion .

GLYCOGEN IN LIVER CELLS :-

- The liver cells are the chief glycogen stores in the body and play an important role in glycogen metabolism . liver glycogen has two main sources:-

1. The glucose carried from the alimentary canal as the end product of carbohydrate digestion . This glucose passes to liver cells by the hepatic portal circulation , and become polymerized in the liver cell into glycogen .
2. The second source is the lactic acid produced in the muscle cells as a result of glycolysis (break down of glycogen)during muscle work . this lactic acid passes from the muscle cells to the liver cells where it under goes polymer – ization into glycogen

- Glucose in alimentary canal Polymeration glycogen in liver cells .
- lactic acid in –muscle cells Polymeration glycogen in liver

* The hepatic portal vein which carried , glucose is branched into a large number of small vessels lying at peripheral region of the hepatic lobules.

- the peripheral cells of lobule are the first receive glucose and therefore they become loaded with glycogen
- Glucose continues to diffuse into the hepatic lobules , but the rat of diffusion is markedly decreased in the inner regions of lobules , this glycogen content is seen to be much less in the inner lobule cells than in outer ones .

- The diffusion of glucose continues until reaches the central vein in hepatic lobule which is small branch of the hepatic vein which leaves the liver and pours to heart .
- Thus , it is clear that considerable amount , of glucose which reaches the liver is stored as glycogen in the liver cells . And the other part reaches to the blood circulation and the body cells where it is oxidized to give energy .

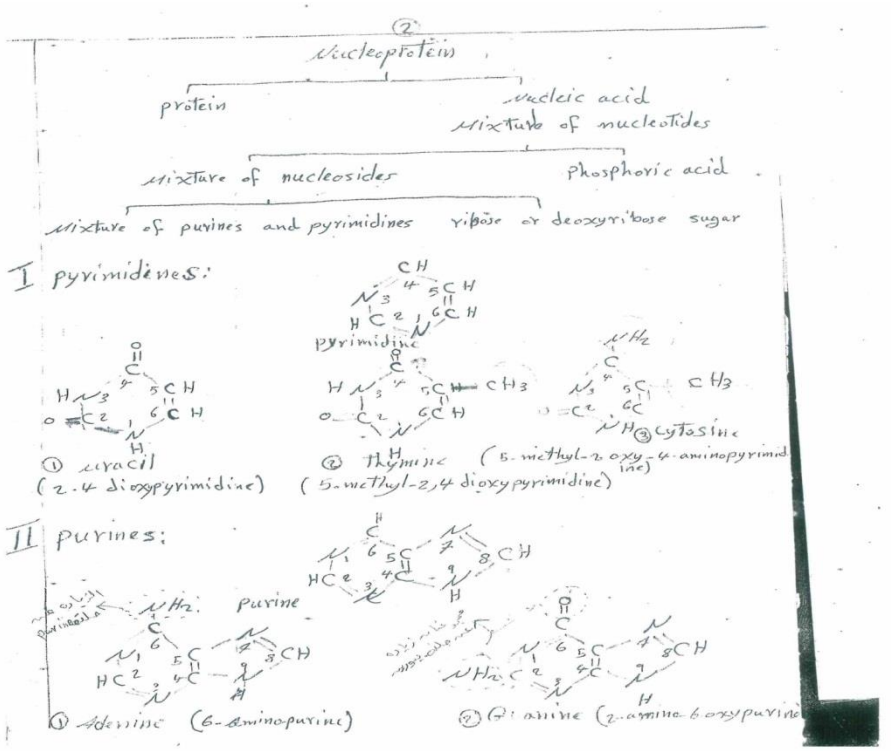
NUCLEIC ACIDS

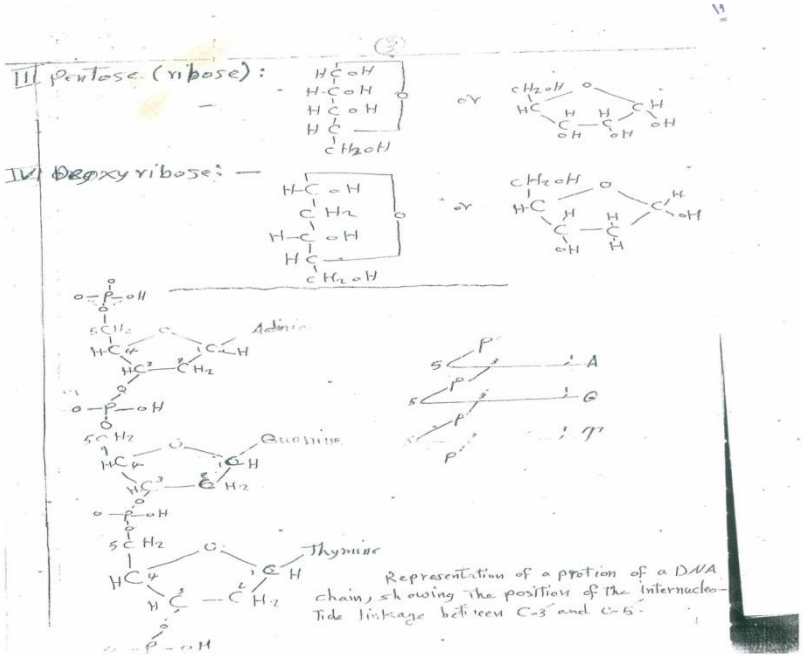
INTRODUCTION :-

- The nucleic acids , deoxyribonucleic (DNA) and ribonucleic acid (RNA) are found in animal and plant cells , and are usually combined with basic protein to form nucleoproteins .
- DNA mainly is found in the nucleus , specifically associated with the chromosomes , although there are few reports that DNA occurs also in the chloroplasts and even in the cytoplasm .
- RNA is found in the cytoplasm and a small nucleic acids ; DNA and RNA yields the following components :

(a) Phosphate groups (b) five – carbon sugars and
(c) Nitrogenous bases , purines and pyrimidine's
Every one of these type are two Bath DNA and RNA have this basic structure but differ in two important aspects
These are :
(a) Sugar content : in DNA the 5-carbon sugar is deoxyribose but in RNA it is the ribose .
(b) Purine and pyrimidine content :

- in DNA the purines are adenine and guanine , and the pyrimidine are thymine and cytosine
- In RNA the purines are the same as in DNA but the pyrimidine's are uracil and cytosine.


Nucleoprotein
protein
nucleic acid
mixture of nucleotides
mixture of nucleosides
phosphoric acid
mixture of purines and pyrimidines
ribose or deoxyribose sugar
I pyrimidines:
pyrimidine
① uracil
(2.4 dioxypyrimidine)
② thymine (5-methyl-2,4 dioxypyrimidine)
③ cytosine (5-methyl-2 oxy-4-aminopyrimidine)
II purines:
purine
① Adenine (6-aminopurine)
② Guanine (2-amine-6-oxypurine)


III Pentose (ribose):
or
IV Deoxyribose:
or
Adenine
Guanine
Thymine
Representation of a portion of a DNA chain, showing the position of the Internucleotide linkage between C-3' and C-5'.

- MOLLECULAR ORGANIZATION OF DNA From the cytochemical studies and x-ray diffraction data, Watson and crick (1953) proposed a molecular model which is widely accepted as representing the natural stat of the DNA molecule. In other words , cytochemical studies of DNA obtained from a variety of cell types indicate that : the amounts of thymine and the amount of guanine always equals the amounts of adenine and the amount of cytosine respectively.
- THIS PAIRING OF THE NITROGENOUS BASES TOGETHER E x-ray diffraction data are the bases for the Watson & crick working model of DNA.
- They have proposed a double helical structure in which the two polynucleotides are collide in such a manner that adenine of one chain is bonded by hydrogen to a thymine of the other, and a guanine of one chain is similarly bonded to a cytosine of the other.

BIOLOGICAL SIGNIFICANCE OF NUCLEIC ACIDE:

Role of DNA in genetics:

- It is generally accepted that DNA is the hereditary material : when pure DNA , extracted from a particular strain of bacteria characterized by a special feature , (e-g having a sugar – containing capsule) was added to a culture of another strain of bacteria that lacked this feature , many number of the second strain a acquired this character forever and transmit it to their progeny .
- This means that the given DNA was incorporated with the original DNA .
- It has been suggested that the genetic or hereditary material of the cell must have 2 separated functions :

1- It must be capable of self-duplication

2- It must be capable of initiating certain action that are ultimately exposed in a given cell structure or function

- The expression of the gene action is the formation of a protein which may be an enzyme of structural protein DNA must be therefore capable both of duplication itself and of

providing the necessary information of protein synthesis the structure of DNA make it easy

INTER-RELATION BETWEEN DNA AND RNA:-

- It has been that both the nuclear and cytoplasmic characteristics of a cell are determined by the genetic material of the nucleus , namely the chromosomes in other words , the synthesis of specific cellular protein , enzymes and cellular structure is determined by the information in the games .
- HOW ARE THE INSTRUCTIONS IN THE CHROMOSOME TRANSMITTED TO RECIPLONT PROTEIN SYNTHESIS

CYLOPTASM 2 THE ANSWER IS AS FOLLOWING :-

- The nucleus has , of course , some of the same reaction activities as the cytoplasm :

1- it can make its own energy in from of ATB , for it seems to have many of the glycolytic enzymes

2- It can synthesize various small molecules , and it has various enzymes to perform different vital functions .

- This means that , the nucleus has its own comp lament of enzymes ; and these enzymes must be present in the nucleus of the daughter cells .the DNA of chromosomes has specification for the synthesis of these enzymes . But most of the enzymatic material , and much of the cellular synthetic mechanism is in the cytoplasm and the information for the duplication of the material is in the chromosomes .

- There is no direct production of protein by genetic DNA , but there is an intermediary substance which constitutes a line between the genetic information and the specific protein , this substance is the RNA which has the ability of controlling the synthesis .

ROLE OF RNA :-

- It is clear that the means of information between DNA in the nucleus and the synthesizing machinery in the nucleus and cytoplasm is tied up with the metabolism of RNA .
- RNA is found in nucleolus , connected with chromatin as curtain fraction and in cytoplasm as small particles (ribosomes)
- Different experimental procedures have led to the hypothesis that RNA is synthesized in the nucleus and that the completed RNA molecules then move out into the cytoplasm.

TYPES OF RNA :-

- There are 3 main types of RNA these types :-

1- ribosomal RNA (r-RNA)
2- messenger RNA (mRNA) or informational RNA
3- transfer RNA (t-RNA)

- These different types are similar in the presence of A, G, C and U but some are double stranded as DNA and other appear single stranded .

ACTION OF RIBONUCLEASES AND DEAXYRIBONUCLEASES ON NUCLEIC ACIDS :-

- The highly polymerized nucleic acids or polynucleotides as they exist in nature are broken down into smaller comp – potent by the action of specific enzymes for examples :-
- ribonucleases (RNase) split RNA
- deoxyribose nuclease (D nose) split DNA

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HISTOCHEMICAL DEMONSTR OF DNA &RNA

- For the identification of DNA and RNA the following HISTOCHEMICAL METHODS ARE EMPLOYED .

1- feulgen method for DNA
2- methyl green – pyronin method for RNA , DNA
3- gallocyanin – chrome alum method for RNA, DNA

4- NAH- feulgen method for DNA,
5- acridine orange method for RNA, DNA
6- deoxyribose nuclease extraction for DNA,
7- rib nuclease extraction for RNA

GENERAL REACTION FOR NUCLEIC ACIDS

REACTION FOR ORGANIC PHOSPHATE :-

- The phosphate radical is demonstrated in DNA of the nucleus by hydrolysis and subsequent fixation of the released po4 groups with ammonium moly date where the phosphor moly date is formed .
- The resulting phosphor moly date is reduced to a blue compound with benzamine .
- Hydrolysis occur by using either enzyme hydra lyses with nucleases or prolonged hydrolysis with N-HCL .
- This method use to demonstration the presence of po4 in the chromosomes , but is not very suitable for general use Histochemistry .

REACTION FOR DEOXGRIBOSE AND RNBOSE

- In 1944, the scientist given a method for the detection of DNA and RNA depending on mild acid hydrolysis following by reaction with phenyl (or methyl) trihydroxy fluorine using methyl substituted fluorine , DNA stained violet to blue black and RNA yellow to red.

REACTION FOR PURINES AND PYRIMIDIMS :-

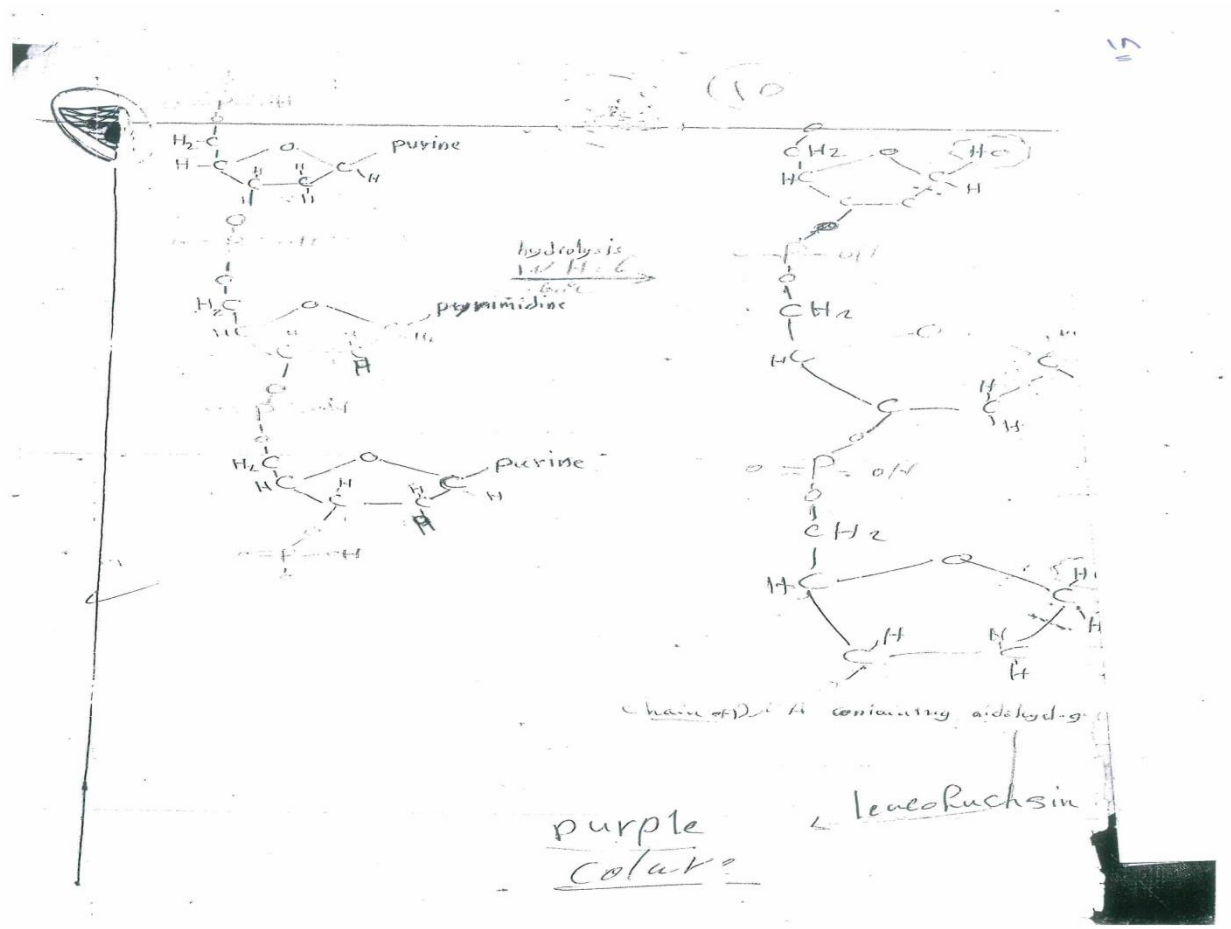
- The tetrazonium reaction after benzoylation or acetylation is the only reaction which has used for the histochemical demonstration of purines and pyrimidine's :-
- Barnard and Danielle (1956) have made a comprehensive study of the coupled tetrazonium reaction in relation to nucleic acid and claim that the reaction is specific for (protein in a special form of combination with nucleic acid) the reaction can use for the identification, the reaction useful

for demonstrating histidine – protein not only in nucleoproteins but also in many other types of protein

PONCTIONS SPECIFIC FOR DNA AND RNA

1- THE FEULGEN SPECIFIC

- This reaction depends on the treatment of fixed tissues by mild acid hydrolysis (with - n – HCl at 60 c) which could removes the purines and liberates the aldehyde groups from the deoxyribose sugar of DNA
- Following hydrolysis , the tissue are washed and then trans ferried to Schiff's reagent which react with the exposed aldehyde groups to produce a purple dye in the nuclear chromatin .
- The specificity of this reaction become the object of creasing doubt DNA purple dye .
- Other another's shown that , the specify of feulgen reaction depend on the ability of N – HCL ,to brock down the bound (linkage) between C and C^2 which found in deoxyribose only and not in ribose . Therefore , the aldehyde group forms only in DNA and not in RNA.



2- METHYL GREEN – PYRONIN FOR DNA AND RNA :-

(The Unna pappenheim stains)

- This technique is used for demonstrating both DNA and RNA. It was first published by pappenheim (1894) and modified by Unna.
- Methyl green is an impure dye containing methyl green must be wash with chloral – form until remove all the violet.
- This dye is a basic dye and specific for DNA at a slightly acid PH .
- Its affinity for DNA is not fully understood kurnick (1955) has suggested that . Binding of methyl green to DNA involues two sites , two amino groups on the dye combining with two phosphoric acid groups of the DNA .
- Pyronin , is less specific and the PH of the stain – LNG is critical . The chemical basis of the method is not fully understood . it is advisable to use a control section that subjected to RNase results :-

DNA : green RNA : red

3- GALLOCYANIN – CHROMATUM FOR DNA AND RNA : (GC)

- This method is specific for DNA and RNA and more reliable than methyl green – pyronin . The (GC) is a pro - aggressive stain and it withstands alcoholic dehydration and clearing in xylene .
- Staining can be carried out at any PH between 0.8 and 3.3 but it is better between PH 1.5 and 1.75

4- FEVLGEN – NUPHTHAIC ACID – HYDRAZIDE REACTION :

- For DNA this method is as a control method for the feulgen nuclear tech Different result will not be stained by this method .
- The section are hydrolyzed by using N.HCL the free aldehydes produced combine with 2- hydroxyl 3 – naphtha acid hydrazine . This is then coupled to fast blue B produce a purplish blue color at the site of coupling .
- The localization is identical to that shown by the true feulgen reaction This method specific for DNA

5- ACRIDINE ORANGE : FOR DNA & RNA

- This fluorescent technique is wide used in cytology to demonstrating DNA ,RNA . Formalin and bouin fixate must be avoided because they prevent differential staining .
- The successful application of this method depends on and the fixative applied .

RESULT :-

- DNA fluoresces apple green
- RNA fluoresces red

6- DIGESTION METHODS :

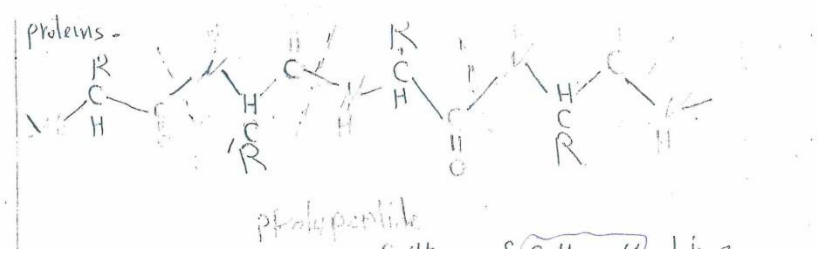
- When deoxyribose nuclease is applied to tissue sections , all DNA is removed whilst RNA is unaltered , because Dnase is specific for DNA

  Also when rib
- Removed and DNA is unaltered , because Rnase is specific for RNA .
- The two enzymes technique for the digestion of nucleic acid are valuable as controls for methods discussed .

Proteins

Proteins and amino acids:-

- Proteins are organic substances of high molecular weight . they are formed by a number of amino acids which , derived from aliphatic acids such as acetic acid (CH3 COOH) by replacement of one hydrogen atom by the amino group (NH_2) Shown by the simplest of these compounds , namely amino acetic acid
- One of the most important characteristics of the amino acids is their capacity of combining with each other to for long chains. This property is due to the presence of the carbon group (COOH)and amino group (basic group NH_2) in each molecule . these substances which contain at the same time acid group
- Proteins are amphoteric ,ice , they possess both acid and basic properties , depending upon the reaction of the solution they usually form colloidal solutions (emulsions) in water .
- Separation of proteins from solution is by precipitation in the presence of electrolyte at low temperature, with alcohol at varying H^+ concentrations . Physical techniques using centrifugal methods or electrophoresis may also be used.
- The condensation of amino acids occurs in such a way that; the acid group of one molecule combines e the basic group of another molecule with the loss of one H_2O.
- When the amino group of one amino acid joins with the carboxyl group (- COOH) of another one and loss one molecule



- Protein are built up of C,H,O,N and in son cases with traces of sulphuric, iron, copes and phosphorus.
- Also, amino acids can be obtained from proteins by hydrolysis under effect of the enzymes or by boiling with strong acids or bases .

TYPES OF PROTEINS :-

- From the biochemical point of view, proteins are of non – proteins nature .
- Which a simple protein is combined with other substance called prosthetic group . examples of this group are :-

a- nucleoproteins : in which the prosthetic group is the nucleic acids

- Ex : nucleon , nucleohistone .

b- Glycoproteins belong to the category of conjugated proteins, The glycoproteins occur in blood plasma, egg albumin, saliva, mucus, and blood group substances.

c- Lipoproteins : consists of lecithin (lipid) and protein .

- Ex : blood fibrinogen .

d- Chromo proteins: in clude a number of colored substances as the respiratory beignets hemoglobin (haene + globin) and haemocyanin (copes + protein). Also respiratory enzyme as cytochromes and flavoproteins.

- DERIVED PROTEINS :

- These include compounds of coagulated protein and the partially soluble proteins as protease , peptones and polypeptides . These proteins which may be isolated of The removal of non – protein prosthetic group of conjugated proteins.

SIMPLE PROTEINS :-

- The simple proteins are classified into various groups according to their solubility's , these group are :-

a- ALBUMINS : soluble in water and coagulated by heat E-g serum albumin

B- GLOBULINS : insoluble in water but soluble in various dilute salt solutions, e-g serum albumin

C- GLOBULINS : soluble in water but insoluble in dilute solutions of ammonia e.g. nucleohistone of thymus gland .

D- Protamine: soluble in water and coagulated by heat.

E- Scleroproteins : insoluble in all neutral solvents , but soluble in acids and alkaline e.g. serum albumin

- From the structural point of view, the simple proteins can be classified into :

- FIBROUS PROTEINS : contain the collagens, retie line , elastin and keratin these proteins are insoluble in acetous media .
- GLABULAR PROTEINS : contain, globulins , histones , proteins and albumins which are soluble in aqueous medium And many of them have been crystallized .
- However proteins which are interest from histochemical point of view as the following :-

- HISTONES :-

- These are proteins characterized with a strong alkaline reaction and they are known as basic proteins because of they are composed of the basic amino acids , arginine , lysine And histidine .
- Some of these proteins unite with nucleic acid Form nucleohistone which are found in large amounts in the tissues of certain organs such as pancreas , thymus gland

- PROTAMINES :-
  - They are having strong alkaline reaction and combine with nucleic acids forming which are the chief constituents of chromosomes .

- COLLAGEN AND RETICULIN :-
  - Collagen consists of bundles of coarse non-bra fibers . These bundles as red stained with venison , faintly pink with PAS and yellow or brown with silver impregnation technique . Collagen contain very sum amounts of glucose , GA lactose and ribose.
  - Reticulin consists of fine branching fibers (network) which are unstained or faintly stained with van Gleeson , deep stained with PAS and black by silver impregnation .
  - Reticulin is as the precursor of collagen and it contain large amounts of galactic glucose and ribose .

Physical characters of collagen and Reticulin :

- Electron , microscope .Studies showed that the collagen fibrils were crossly striated by 650 a cross – striations – they are similar in this respect to the striated muscle fibers.
- It has been found that collagen is composed of bundles of regularly arranged fibrils , Reticulin have a membranous structure in which the fibrils occur in irregular form .

RETICULIN DIFFERS FROM COLLAGEN IN 4 RESPECTS :-

- It consists of a large number of minute anastomosing fibrils lying in an abundant amorphous matrix.
- Are arranged at random rather than in parallel bundles.
- in its membranous structures rather than fibers.
- it not give gelatin on boiling

BIOCHEMICAL FEATURES OF COLLAGEN AND RETICALIN :-

- As regards the amino – acid composition of collage and the product of its partial hydrolysis (gelatin) they show a high content, particularly of glycine , protein , and hydroxyproline
- The amino –acid contents of Reticulin are nearly same as these of collagen except for proline and hydroxyl proline in which Reticulin is somewhat deficient .
- There are three forms of collagen can be described :

1- Acid –soluble collagen and is extractable with Dilute acetic acid

2- the second form can be extracted from the skin with a weak alkaline phosphate buffs or means
3- The third form known as the insoluble – collagen which constituents the bulk of tissue collagen .

- The neutral salt-soluble collagen is regarded as the precursor of the insoluble – collagen .it is believe that during early development, Secreted by fibroblasts and is transformed into collagen fibrils.

RETICULINE WAS FOUND BOUND WITH FATTY ACID

- They contain small amounts of phospholipids .

EFFECT OF TEMPERATURE ON COLLAGEN AND RETICULIN :-

- It was found that, collagen fibers undergo shrinkage on heating in presence of H2O and loss resistance to trypsin and also most of their optical characters.
- It was found that, formalin fixation increases the temperature at which collagen under goes thermal shortening from 65 c to about 90 c

ELASTIC TISSES :-

- Elastic differs chemically from collagen in posing far less arginine , histidine and lysine and more leuc And valine . It contain no tryptophan but has slightly more tyrosine than collage . It contains more lipid than collage .
- Elastic tissue are attached easily by pepsin a small extent by trypsin.
- Marked changes occur in the elastic fibers. These take the form of longitudinal splitting and breaking into fragments and mttineotely into granules. These changes are associated with chemical changes in the amino acid content and in the calcium content of elastic fibers.
- KERATIN :-
- Keratin is a fibrous protein characterized by its high content of the basic amino acids arginine, lysine and histidine and of the sulphuric – containing amino acid cysteine. It has a strong affinity for both basic and acidic dyes and is impervious to the action of pepsin and trypsin.

Kara in hair differs from that found elsewhere in its high values for cysteine and lower values for cysteine.

- Generally; the identification of protein dependent (can by) the identification of amino acid which is mainly of this protein.

GENERAL IDENTIFICATION OF PROTEINS

- The classical methods which have been employed for the demonstration of protein are :
  1- millon's reaction for tyrosine
  2- the diazonium reaction for tryptophan and histidine
  3- the xanthoproteic reaction for phenolic compounds
  4- the Saguache reaction for arginine
  5- the nitroprusside test for sulfhydryl group
- Appositive reaction indicates the presence of protein. all these reactions except the last can be applied to fixed tissue sections but only two(the million and the diazonium reaction) are essentially suitable for the demonstration of protein

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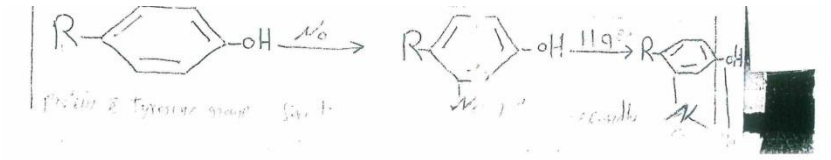
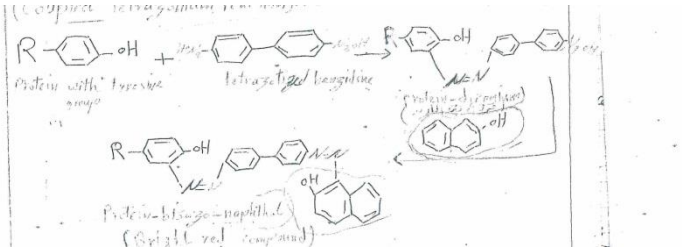
- THE MILLON REACTION :
  - Millon's reaction (1849) is based on the presence of the hydroxyl – phenyl group in the protein molecule. The only know amino – acid containing the hydroxyl- phenyl group is tyrosine.
  - The original millon's reagent was made by digesting mercury in nitric acid and diluting with water, forming mercuric nitrate (Hg [NO3]2).
    1- First, a nitrosophenoteis produced by the substitution of No for H ortho or Meta to hydroxyl of the phenol.
    2- Secondly, Hg^{24} is incorporated into anew ring; by chelation which includes the nit rose group the new complex (result) is red in color.



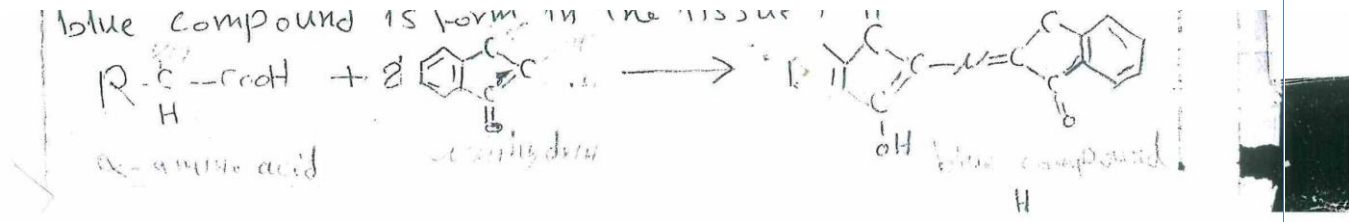
- the diazonium reaction :-
  - Diazonium compounds are prepared by the action of nitrous acid (in the cold) on the salts of primary aromatic amino; sulphanilic acid (p- amino benzene sulphonic acid) is commonly for this purpose. diazonium compounds such as diazonium hydroxides, acting in alkaline a aqueous solution, combine with

1- The phenol group of tyrosine 2- the in dole group of tryptophan 3- The heterocyclic imidazole group of histidine to give colored products.

- The method for the simple demonstration of protein in tissue sections, making use of the above principle is the (coupled tetrazonium reaction) this reaction can be show as



- ninhydrin reaction :-
  - Ninhydrin reacts, with the free NH_2 groups of –amino-acids to give a blue – compound, carbon dioxide and an aldehyde. During the reaction which is carried out at 100 c the bluish violet color develops rapidly and disappear. And the blue compound is form in the tissue.



- MUCOID SUBSTANCES
  - The Mucoid substance :- the Mucoid substances are built up of sugar unites in which a hydroxyl group is substituted by an amino group (NH_2) these compound as known as amino sugar or glucosamine.
  - The Mucoid include three main classes :

1- Muco poly saccharides
2- Mucoproteins
3- glycoproteins

1-MUCOPOLY SACCHARIDES :-

- The muco poly saccharides which contains an acid as uranic acid or sulphuric acid linked . With the carbohydrate units are identified as acidic muco poly saccharides .
- The muco poly saccharides which do not contain acid groups are known as neutral muco poly saccharide .

A- neutral muco poly saccharides :-

- They have height hydration and forms vicious fluids, jellies and solids as mucus, jelly of umbilical card and cartilage .
- A number of this substance accrue as extracellular products as the mucous of the glandular secretions or the matrix of connective and cartilage :

- CHITIN :-
  - Chitin which is the simple neutral mucopolys C-charades and mainly exists in exoskeleton of insects and other arthropods . (Exoskeleton canton less than 5% chitin and the remainder substance are protein or protein and calcium carbonate .
  - Chitin is insoluble organic materials but can be solved by hot HCL, H_2SO_4 and similar cellulose in chemical structure

where it consists of long chains of monosaccharide aide units which are glucosamine unite. But these units are glucose in cellulose .

- Chitin stains by reddish purple with chromic acid oxidation and Schiff's regent .

B- ACID MUCOPOLY SACCHARIDES :-

- These substances are limited to animal sources and are components of all epithelial mucins of an alimentary canal and they found in the majority of duct mucins.
- they are characterized by the presence of an acid which is glucuronic acid as their second carbohydrate compo.

- acid muco poly saccharides are classified into:
- Simple acid muco poly saccharides : such as hyaluronic acid
- Hyaluronic acid is composed of the (glucosamine) and glucuronic acid and found in highly polymerized Diction, thus it acts in presence of Hyaluronidase

hyaluronic acid polymerized $\xrightarrow[\text{Enzymes}]{\text{Hyaluronidase}}$ Hyaluronic

- It protect the tissue from spearing

* COMPLEX ACID MUCOPOLY SACCHARIDES :-

- These are substances consists of :
- Composed of glucosamine, glucuronic acid sulphuric acid .
- It is an anticoagulant substance which prevents blood clotting in tissues.

* CONDROITIN SULPHATE :-

- It components of mammalian connective tissue and cartilage they composed of sulphaled N-acetylgalactosamine joined to glucuronic acid .

2- MUCOPROTEIN :-

- These substances are hexosamine – containing polysaccharide occurs in a firm chemical union e a peptide and hexosamine content exceeds 4% of the total weight.
- Mucoproteins are PAS positive but are negative e toluidine blue
- they give positive result with a [protein method

3- GLYCOPROTION :-

- They are distinguished from Mucoproteins but the hexose amino content is less than 4% .

- glycolipids :- (cerebrosides) Such as phyrenosin and Kerasin and they are found in the tissues of the central system

- They have not phosphoric acid and soluble in pyridine MENERALS .

CALCIUM

- Calcium is the most important minerals in the body which can be demonstrated histochemical .
- Calcium found in different forms such as soluble form (in blood stream) ionized form, insoluble form and masked form .
- Calcium is the most important cation in the bone and present in form slot bone. When ca^{++} increase in the body, it precipitate in tissue which in normally do not contain ca^{++} .

CALCIUM CAUDEMONSTRATED BY :

1- ALZARIN REDS :- This used to demonstration of ca^{++} in the section but not specific for ca^{++} .

- The specify can be increase by performing the reaction within PH6.3 – 8.5 .

2- VON KOSS SILVER REDUCTION METHOD :-

- This method is used for demonstration of phosphate and carbonate and not demonstrate ca^{++} since most of phosphate and carbonate are those of calcium .
- thus , calcium is indirectly demonstrated

IRON

(Perl method, the iron sulphid method)

- Iron found the body bound to protein such as hemoglobin, hacmosidrine and ferritin. Some iron can be separate from protein by using reduction agent such as hydrosulphite. Other iron strongly bound to protein such as in hemoglobin , but treatment by 100 vela , hydrogen peroxide may be release amount of iron .
- Iron can be demonstrated by Perl's method that depend on the production of ferric Ferro cyanide when ferric IOUs in the tissue react with Ferro cyanide that found in acid solution .

*naphtha chrome green B method :-

- The modified method used to demonstration of beryllium, aluminum , iron and calcium .
- This method give with their metals the same color . the PH of stain is critical such as :-

*at PH 5-0 :-

- Deep green is given by beryllium and dye .
- Calcium is soluble .
- iron and aluminum are weakly stained

* At PH 7-2 / 7-4 :-

- Deep green is given by aluminum and dye .
- Beryllium weakly stained .

Calcium :-

1- alizarin red s m
2- von Koss silver reaction

Iron :- Perl's method

Copper: - rubeanic acid techno

Lead :-

1- supplied silver – method
2- rhodizonate method red

Beryllium :-

1- Solo chrome asinine m .
2- Naphtha chrome green m .

Aluminum :-

1- Solo chrome
2- Naphtha chrome green

- The lipids are organic substances which are inside Mble in water, soluble in fact solvents. The lipids include fats, oil, waxes, and related compounds.
- In this chapter , the term (lipids) will be used to clude all naturally occurring fat and fall like malaria while have formerly known as lipoid, lipin, lipine and libido- lipid Normal components of all Tissues and are usually in the form of stored lipid or as particular lipids structures such as myelin
- Lipids are important constituent not only because of their high energy value but also because of the vitamins and

Essen fatly acids which are associate will the fat of energy and as an insulating material in the subcutaneous tissues and around certain organs .

- most lipids are compounds soluble in organic solvents and for this Eason , many of the histochemical techniques are applied to frozen sections lipid are bound to protein (lipoproteins) on carbohydrates (glycolipids) with the resulting cha of their chemical and physical properties.
- A great proportion of lipid present in tissues are associated with proteins , thus two types of lipids are histochemical in dentil

1- A visible lipid is easily demonstrated in the cell and tissue by the specific lipid method as the Sudan dyes, and Nile blue sulphate.
2- Invisible or masked lipids which could not be demonstrated directly following such techniques such types are found in normal condition combined with pro.

The following:

1- lipids
2- compound lipids
3- derived lipids
4- carotenoids

simple lipids :

- These are esters of fatty acids with alcohol ,the include the following .

A-Glycerides. B-waxes .

A-GLYCERIDES:

- These often are called triglycerides or neutral fats
- these are very important from the histochemical point of view.
- they are esters of fatty acid and glycerol .glycerol is a trihydric alcohol; its formula is cH_2Oh CH OH cH_2oH
- Glycerol can react with 3 molecules of an acid to form triglyceride. E. g; it can be react with butyric acid to form a triglyceride (tributyrin) which is the simplest fat.

CH_2oH Hoocc3H7 cH_2-$ococ_3H7$

CH OH + $Hoocc_3H7$ $CH\text{-}ococ_3H7$

CH_2OH $Hoocc_3H7$ $CH_2\text{-}ococ_3H7$

- Where R varies according to the fatty acid.
- in animals , the most important fatty acids combined with Glycerol are palmitic , stearic and folic acid . All these acids IS this group belong the fats and oil
- These are esters of fatly acids with alcohols other than glycerol, e.g. Bee wax.

compound lipids;-

- These substances consists of a fatty acid, an alcohol (which is usually glycerol) and one or several additional groups. These include the following compounds .

1- phospholipids
2- Glycolipids.

1- phospholipids :

- These substances ,on hydrolysis , yield fatty acids + glycerol (or some other alcohol) + phosphoric +acid +a base such as choline or serine
- these substances occur as essential Constituents of the protoplasm

$cH_2o\text{-}co\text{-}R$ CH_3 $Ch_2\text{-}ocoR$

CH o-Co R cH_3 CH-o-co R

$cH_2o\text{-}p\text{-}o\text{-}cH_2\,cH_2$ N Ch_3 $Ch_2\text{-}o\text{-}$ $\text{-}cH_2\text{-}cH_2$

- the chief this phospholipids includes 4 other groups which are :

1- phosphatidly inositol (lipositds)
2- Plasminogen.
3- phosphatidly serine (kephalin)
4- Spingomyelins (phosphatidly sphingosides.

1- phosphatidly inositol (lipositols) :

- Inositol as constituent of lipids occur in acid –fast bacteria , in phospholipids of soybeans and of brain tissue as well as in other plant phospholipids.

2- phosphatidly serine:

- Such as Kephalin the amino acid serine rather than ethanol amine It is present in brain tissues

3- Plasminogen :

- These compounds constitute about 10% of the phospholipids of the brain and muscle structurally, the plasminogen resemble lecithin and kephalin , but give appositive reaction which tested for aldehydes with Schiff after fixed the phospholipid with mercuric chloride

4) Spingomyelins (phosphatidly sophoroside)

- They are found in brain and nerve tissues. No glycerol is present . on hydrolysis , they yield a fatty acid, phosphoric acids choline and complex amino alcohol (sphingosine)

2- Glycolipids : (cerebrosides)

- They contains fatty acids , acorbohydrale (glucose Gala close) and comply alcohol such as sphingosine but no phosphoric acid .

*Derived Lipids :

- These are substance derived from the above groups by hydrolysis they includes fatty acids , glycol , stereo and other steroids , alcohols other than glycerol and sterol , fatty ,aldehydes and proteins of lipoproteins .

A-fatty Acids:

- Fatty acids are obtained from the hydrolysis of fats . they includes saturated acids or unsaturated.

- saturated acids :
  - These are theoretically built up on acetic acid as the first member of the series . the general formula is For cn H2n cooh for example butyric acid ,palmitic , stearic ,……..etc.
- unsaturated fatty acids :
  - These may be subdivided , as to degree of unsaturation ; into the following .

1- oleic series : one double bond . general formula :$CNH_2N_{-1}COON$
2- linoleic series: tow double bond . general formula :$CNH_2N_{-3}COOH$
3- linoleic series :there double bond . general formula : $CNH_2N_{-5}COOH$
4- fatty acids: with 4 double bond ,$CNH_2N_{-7}COOH$ and with 5 double bounds $CNH_2N_{-9}COOH$

B-Alcohols:

- Alcohols ,in the lipid molecule. Glycerol cholesterol, and (the higher alcohols(e .g Pinacolyl $C_{16}H_{33}Oh$)
- usually found in the wax the presence of glycerol is indicted by the acrolein

C-fatty Aldehydes:

- The fatty acids may be reduced to fatty

aldehydes. These compounds are found either combined or free in natural fats.

D- steroids :

- The steroids are often in association with fat.
- They may be separated from fat after he fat is saponified .
- These include a number of highly important substances in the Body such as the sex and adrenal hormones ,vitamin D, bile acids ,etc…..
- Steroids possessing "OH" group are called sterols of these compounds cholesterol which is widely distributed and the principal constituent of the wool fat. Cholesterol is found in the bile , brain. Adrenal gland and other organs it know as 3-hydroxyl-5,6-cholestene.

position:

- Glycerides: serve as stores of energy and may be provide a protection against cold and injury.
- Lecithin : is believed to play an important role in the metabolic actives in lives.
- Phospholipids and cerebrosides: are found in the nervous tissue.
- Cholesterol: is important in regulation the mechanical properties of epidermis and hairs.

- fat is present in most tissues as in the whereas, the lipids of the tissue consists of Neutral fat and Phospholipids, the first is stored fat but the latter is present as constituents of the structure of the cytoplasm.
- In the other words, is clear that , fat are grouped into.

1- constant fat :these are mainly phospholipids and do not dis . appear during fasting .
2- variable fats : these represent the depot ones which by the fasted animal .

- Generally , the liver lay an important role in the fat metabolism, the normal lives contains about 4% Of lipids of which 25% is essential fat and 75% are phospholipids, may be phospholipid nucleoprotein complexes which play role In cellular metabolism

*Demonstration and identification of lipids lipid material :

- Lipids are usually present as granules as in some cases bound to components within the cell . lipids are difficult to demonstrate as they form complex groups with other substance e. g with protein to form lipoprotein the physiochemical state of lipids in cell and tissue is rarible.

fixation of lipids:

- Is preferable , sometimes , to demonstrate lipids in tissues sections. Fleming – without acetic acid , Regard fluid an . Aoyama fixative were better employed for the fixation of lip material- phospholipids state of lipids in cells and tissue is rarible.

fixation of lipids:

- it is preferable sometimes , to demonstrate lipids in tissues sections. Fleming
- Aoyama fixative were better employed for the fixation of lip material- phospholipids may be lost in formal saline and using calcium chloride, because of its buffering effect, stops the loss of phospholipids.
- The majority of lipids, including triglycerides and most phospholipids soluble in alcohol . Alcohol. Fixation on makes sectioning difficult and should be avoided.

- Mercuric chloride fixative reacts with phospholipids and makes the blocks brittle and difficult to cut . osmium tetroxide
- Fixative react with double bonds of unsaturated fatty acids and using in number of histochemical methods for lipids.

Demonstration of lipids:

- Lipids can be demonstrated by using physical methods ,with and without dyes, and histochemical techniques .
- it should be noted that:

1- the compound lipids contain some hydrophilic lipids which are water soluble; some of these of these can be changed insoluble by fixation in formal. Calcium
2- the simple lipids are soluble in many organic solvents and for their demonstration frozen section must be used
3- some lipids are liquid at body temperature but may be solid at room temperature with will affect their staining properties .
4- Also many lipids found in tissue are bound to proteins or carbohydrates, with the resulting change of their chemical and physical properties.

Extraction:

- These methods are to demonstrate specific tissue lipids because of the binding of binding of lipid to other tissue structure . process

1- 1)fresh tissue blocks in the extraction fluid (solvent) Or fluids for up to 48 hours at 60 c . or at the boiling point of the solvent . the block is fixed in formal saline for hour.
2- wash in tap wastes , frozen and suitable sections are cut.

- These are many extractive agents or solvent as
- cold acetone removes (extract) glycerides , cholesterols and ketosteroids.
- hot acetone removes cerebrosides.
- hot these removes lecithin and kephalin.
- hot chloroform removes all the lipids.

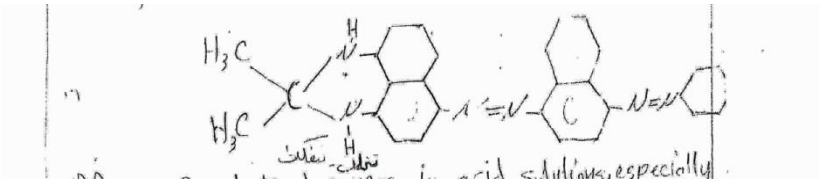
3- extracted – fresh sections are stained alongside un extracted one as a suitable control

General lipid staining methods

Sudan 3 , Sudan 4 , Sudan black B and oil red o have long been used to demonstrate lipids, Sudan black B and oil red o are the most used stains .

- SUDAN BLACK B :- (SBB)

- SBB is a slightly basic dye and combines with acidic groups in compound lipids . it also is a fat soluble dye which has a great advantage in histochemical detection of phospholipids.
- SBB has a slightly different chemical structure from oil red dissolve in triglycerides , but SBB has a greater affinity for other lipids , such as phospholipids



- SBB was found to decompose in acid solutions , especially below PH4 and it is not specific for phospholipids ,but it gives them a positive reaction after the extraction of neutral fats .
- SBB is better employed as a saturated solution in propylene glycol in which . it is much more soluble in alcohol or acetone in such case , SBB or Sudan 4 stains the fat particles much more intensely and besides, a large number of phosphatide – containing structures including mitochondria and microsomes are also intensely stained
- SBB was also found to be a metachromatic stain . when stained preparations, were viewed with polarized light , spingomyelins and Ganglioside produced metachromasia (the color is red instead of black), whereas , cerebroside and cholesterol did not . in case of lecithin, the metachromasia is sometimes so strong that it can be seen with the ordinary light .

ENZYME HISTOCHEMSTRY

Enzymes :-

- Enzymes may be defined as chemical substance

of organic nature produced by living organisms. They are thermo able and capable of increasing the velocity of a chemical Reaction without being used up in the process , or becoming a part of the product formed-in other words ,an enzyme is a biological catalysts, or enzymes are protein catalysts for Becoming the chemical reactions that occur in living Cells-

- The enzymes are necessary for the normal Metabolic processes within the tissue. By catalysts they increase the rates of nearly all intracellular chemical reaction Without Catalysis by enzymes, these reaction would take Place so slowly Than the life as we know it would not be Possible for almost every organic Compound that occur in and for many inorganic compounds also) there is an enzyme Capable of reacting with it and bringing about some chemical changes.
- The enzymes are highly specific catalysts Generally for single chemical reaction. In other words, the Most significant property of enzymes is the high degree specify for thus substrates.
- Enzymes-catalyzed reactions, are to some extent eversible. within the living cell, however, maybe not in fact occur because reaction products are promptly removed.

Coenzyme:-

- Many enzymes catalyze reactions of their substrate Only in the presence of a particular non protein organic compound called the coenzyme. unless both enzyme and coenzyme.are present, no catalysis takes place
- Coenzyme often contain B vitamin (as part of their structure thus many enzymes Concerned with the metabolism of amino acids require enzymes containing vitamin B6.
- The B vitamins nicotinamide, thiamine, riboflavin ,Pantothenic acid, and lipoid acid are important constituents of Coenzymes For biological oxidations and reductions

- The Coenzyme may be known as really as a co substrate. for example - In dehydrogenation reactions, for every molecule of substrate that is oxidized
- one molecule of Coenzyme is reduced

Intracellular Distribution of Enzymes:-

Generally speaking, the enzymes may occur:-

1- free in living Cells, for Example of these enzymes are pepsin, trypsin, lipase, and catalase the last 3 are found in great amount in the liver.
2- Associated with large particles in Cell, e. g cytochromes oxidase, which are accumulated in the mitochondrial membranes, and
3- enzymes associated with other cell structures such as the chromosome nucleic and cell surface The histochemical approach examines the distribution of enzyme activity in a tissue or cell in its naive state:

- Thin (2 to 10m) Frozen section of Tissue, treated with a substrate Particular enzyme.
- In regions where enzymes is present, the product of enzymes _ catalyzed is formed.
- if the product is colored and insoluble, it remains at the site of Formation and serves as a marker for the localization of enzyme.

Principles of enzymes Histochemistry:-

- Enzymes Histochemistry is based on the function of enzymes That they are able to Catalyze a certain specific chance Reaction the reaction, products, of enzymatic activity is taken as an indicator of the enzyme activity; The reaction product shovel

be Colored or colorless.

the reaction can be represent as following

$$AB \xrightarrow{\text{enzyme}} A+B \qquad B \xrightarrow{R} BR$$

AB is the substrates which the enzyme acts A+B the reaction

products; R is the reagent; and BR is the final colored product to be visualized by the microscope.

*In any histochemical enzyme reaction :-

- the substrate should be acted upon by only one enzyme or group of related enzymes.
- the substrate should be sufficiently stable and soluble solution
- the final compounds should be stable and insoluble in water.

*special precaution of Enzyme Histochemistry :-

- there are a number of difficulties special to histochemical work that must be noted as the following :-

- Enzymes are heat labile, the use of techniques that take account of this condition is essential; employed. (to avoid this difficult, frozen sections are used
- Many mitochondrial enzymes are removed or destroyed by normal histochemical Fixation but these enzymes are much Sensitive to the effects of freezing and thawing. Also the use of hypertonic protection media such as polyvinyl can be prevent the damage of mitochondrial enzymes
- lysosomes, which contain much hydrolytic enzymes, are damage by the freezing and thawing of tissue and the enzymes will diffuse from the damaged organelles; this is the main Call of the diffusion seen in post-fixed section of, say acid phosphatase. Thus careful fixation of tissue and treatment in gum sucrose before incubation dose help to retard this diffusion.
- Some of the enzymes are more resistant to damage than other enzymes; alkaline, phosphates is more able to withstand the effect of fixations than acid phosphatase.
- the dehydrogenases have to be demonstrated in unfixed section but a protection solution is used.

Generally: two things are of extreme important these are

1- the preservation of the maximum possible amount of enzymes activity

2- and the maintenance of the in vivo localization

of the enzymes

factors affecting enzyme activity :-

- The fundamental theory underlying all chemical reaction is the collision theory, which means that for two macules to react with one another they must first come in Con or collide with each other - According to this theory, anything that increases the frequency of collisions between molecule will increase the rate of their reaction. the reverse is factors affecting enzyme activity are:

1- temperature
2- pH
3- inhibitors
4- activators
5- Concentration

*Techniques of demonstration of enzymes :-

- The basic principle in the majority of the Techniques of enzyme Histochemistry is that an enzyme in the tissue is presented with its own specific substrate in the incubating medium and a reaction takes place. the immediate product of this reaction (primary reaction product) is frequently Invisible and must therefore be allowed to couple with another substance so that: an insoluble and visible Final product is produced at the site of the enzyme activity.

There are four basic techniques for demonstrate of enzymes

1- Metal precipitation techniques
2- Simultaneous Coupling using diazonium salts
3- Post-incubation Coupling using diazonium salts
4- Self-Colored substrate

*Metal precipitation techniques:-

- this technique is usually used to demonstration phosphatases .the phosphate ions released, combine with a suitable metallic cation (mostly calcium and lead) to produce an insoluble fraction of metal phosphate.

- the phosphates produced are usually in but can be rendered visible by treating with ammonium sulphid thin where a black sulphid is produced - this Technique Can be Consider as a type of simultaneous Coupling, by using metallic ions instead of diazonium salts.

* Simultaneous Coupling:-

- This method used to demonstration of phosphate and this occurs when incubation mixture Containing a substrate and a diazonium salt are applied to suitable sections in a buffered solution.
- The enzyme present in section hydrides The substrate to form an (invisible primary reaction product (PRP) this immediately coupled with diazonium salt to produce the final reaction product (F-R-P) which is visible . this type of reaction such as the azo dye method for phosphatases .
- the substrate must be soluble in water or the buff medium. Also, the amount of the substrate and diazonium salt.
- the inception mediums is important because too much of either Cause inhibition of both rate of hydrolysis and the formation of the FRP.

* Self Colored substrate:-

- there are few methods using this type of procedure.
- The substrate is a colored substance But Soluble, and the effect of the enzyme to be demonstrate is to remove the solubilizing group without interfering with the color. the PRP is, therefore, Colored and insoluble and no Coupling stage is necessary. The insoluble colored reaction product is precipitated at the sit of enzyme.

5- Post-incubation Coupling :-

- This coupling applied only to the large group of azo dye methods. this type of reaction is based on the supposition that" a PRP is sufficiently insoluble will remain in situ without

diffusion either during incubation or during Subsequent performance of the necessary demonstrating reaction .

In this procedure :-

1- the enzyme hydrolyses the substrate and product as reasonably insoluble PRP.
2- the subsequent coupling Carried out in a separate solutions.

this method principle has many theoretical advantages:-

1- this method depends upon the PRP remaining at the first sit the hydrolysis
2- the diffusion of PRP does not occur during the coupling.
3- The optimal PH of the first substrate-enzyme reaction can be occur in the first incubation and a possibly different opt PH For the coupling can be produced in the second coup Stage)
4- This method is applied to histochemical localization , acid phosphatase, sulphate, B- glucuronidase, and glucosan.

Phosphatases :-

- The phosphatases are hydrolytic enzymes, le they belong to hydrolases. They catalyze the transfer of an exchangeable group to water a cording to the following

$$R\text{-}R+H_2O \xrightarrow{\text{hydrolase}} R\text{-}OH+R\text{-}H$$

*The phosphatases are divided into 3 types :-

1- mono phosphatases : hydrolyzing ester with one phosphate group.
2- hydrolyzing ester with two phosphate group.
3- Trip phosphatases: hydrolyzing ester with three phosphate.

General characteristics :-

- phosphatases occur in animal and plant tissues, and are responsible for the hydrolysis of organic phosphate ester
- Some of these enzymes act on substrates which are different from one another in various chemical characteris ,Other

enzymes of the same group are said to be specific act on only well-defined striates .

- the phosphatases in which the substrate spec is less limited, are divided into two groups:

1- alkaline phosphates those exhibiting optimal active at high pH values at ph. g .o or above.

2- acid phosphates; those exhibiting optimal active at low pH values. at region of pH5.0

- Reliable histochemical methods are available the demonstration of Alkaline phosphatase, Acid phosphates ,Nucleotides, Glucose ,phosphatase and Adenosine triphosphate.

*The hydrolysis of organic phosphate esters during Incubation provides the basis for the histochemical reaction:-

- the released phosphate ions or the remaining organic residue Is made visible by variety of means :

1- phosphatase are generally precipitated as insoluble salt such as lead or calcium phosphate which is then converted Into colored sulphid that can be seen under the micros

2- Alternatively, the alcoholic residue of the substrate may be to reaction with a suitable diazonium salt to produce a high colored insoluble azo dye.

- The pH of the final incubating solution is critical- each enzyme has optimal pH value and the controls sections will be used .

ALKALINE PHOSPHATASES

- Alkaline phosphatases can be defined as an enzyme which liberates phosphoric acid from phosphate ester

$$\text{R-o-p-O.H} + \text{HoH} \xrightarrow{\text{Phosphatase}} \text{R-OH} + \text{phosphoric acid}$$

- These enzymes are activated by magnesium and manganese ions. Cyanide and cysteine inhibit alkaline phosphatase activity. exhibiting optimal activity at high ph.

*Histochemical Detection of Non-specific Alkaline phosphate:

- there are many types of histochemical methods available for demonstration of these enzymes such as :

1- gomori (1939) calcium phosphate method (metal substitution)
2- direct lead method (el-Aaser , hassanein , 1973)
3- azo dye methods :there are two methods.

*Gomori (1934) Calcium phosphate method (metal substitution)

- In this method sodium glycerophosphate is used as organic phosphate ester. It depend 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 presence of Ca lens [e. g- Ca nitrate],at PH

(PH around 9.4 is Kept by The Sodium vernal buff) and In presence of magnesium ion as activators .

- the method can be summarized in the follow

1- if a sections is placed in an incubating solution containing
- a substrate , e.g. sodium-B-glycerophosphate.
- Calcium ions e-g. calcium nitrate.
- an activator for phosphatase e-g magnesium chloride.

a precipitate of calcium phosphate is formed at the site of enzyme activity. The enzyme liberates phosphate from the B-glycerophosphate and this then combines with calcium ions form calcium phosphate

2- Calcium phosphate Treated with 1% cobalt nitrate to produce c phosphate this section then washed in D. water to remove the excess of silver nitrate .
3- Sections treated with dilute ammonium sulphid to form Black Cobalt sulphid. which is visible under micro

- Sodium B-glycerophosphate + Alkaline phosphatase → phosphate.
- phosphate ions + Calcium ions. ⟶ calcium phosphate
- calcium phosphate + Cobalt ions. ⟶ Cobalt phosphate
- Cobalt phosphate + sulphid ions ⟶ cobalt sulphid

4- The incubating medium is prepared immediately before used
5- the time of incubation varies with the type tissue used

* direct lead method

- In this method sodium gluecerophosphat is used as organic phosphate ester. It depend on the deposition of lead phosphate at sites of enzyme activity , when sections are incubated with an organic phosphate ester In presence of lead ions [e .g-lead nitrate], at pH 9-5 [PH around 9.5 is kept by the Tries-maleate buffer], tartrate as a chelating of lead (tartaric acid] and magnesium ions as activator-lead phosphate can be visible lead sulphid (dark brown)
- Phosphate ions + lead ion's ⟶ lead phosphate (precipitate)
- lead phosphate + sulphid ions. ⟶ lead sulphid (dark brown)
- The advantages of method ; without lead and magnesium.

1- 1-The incubation medium can be stored in stable stock solution
2- lead and magnesium can be added immediately before Mse
3- the medium is stable for more than 24 hour at room temp or at 34
4- sharp location of the staining was obtained and excellent results have been obtained with different types of tissue that show high low level of enzymes activity
5- using this technique, enzyme can be demonstrated Cytochemical By e .m in different types of Cells.

- azo dye methods

1- Simultaneous Coupling method

- In this method Calcium-naphtha phosphate as Substrate, together with suitable diazonium salt are used. The incubating medium is buffered to g
- The enzyme liberties oc –naphtha From the substrate and this is subsequently coupled with the diazonium salt to form an insoluble azo dye at the sites of enzyme activity.
- this depend on the choice of suitable diazonium salt and pH of the incubating solution.
- Ph. is wide range.
- Diazonium salt e . g fast red TR, fast blue RR.

- The incubating medium should be prepared immediately before and the time is (30 m) at 37c.

2- Azo dye pest- Coupling method:

- In this method, Coupling with diazonium salt take place separately after incubation.
- thus, the product of enzyme hydrolysis should be insoluble and should be remain at the site of enzyme activity during the washing that follow incubation. The section transfer from the incubating solution washed in D. water and then placed in the diazonium salt solution, the product coupling with diazonium salt to farms an Azo dye precipitate at the site of enzyme activity.

Advantages of this method :-

- no inhibitors of diazonium-salt
- PH is wide range and do not depend on optimal PH of enzyme
- no diffusion of the final reaction product with long incubation.

*specific Alkaline phosphatases

a) 5- NUCLROTIDAES

The 5-nucleotidases are enzymes which catalyst
the hydrolysis of nucleoside 5-phosphates giving nucleon
and inorganic phosphate.

5-nucleotidase

nucleoside 5-phosphate ⟶
nucleoside +inorganic pho

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- the enzyme has pH.7.8 to pH g
- the histochemical method for 5-nucleotidases to that of alkaline phosphatase, with the exception substrate which is different in both methods
- the substrate used is adenylic acid (adenosine-5-phosphate)

B) Adenosine Triphosphates (ATPOSE)

- this enzyme is responsible for breakdown of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) with release of energy.
- histochemical methods is very similar to that of alkaline pho but the substrate is Sodium adenosine triphosphate
- Also, the capture of the phosphate ions is made by Calcium or lead Sodium.

C) Glucose-6-phosphatase:

- This enzyme catalysis the cleavage of Glucose 6-phosphate into Glucose and orthophospharic acid.

- histochemical method for this enzyme is Gomori type procedure with lead nitrate at PH(G5-67)
The enzyme is very Thermo sensitive and quit rapidly-inactivated by acid.

*Non- specific Acid phosphatases

- They are widely distributed throughout the bod
- They are found in great amounts in kidney , prostate , spleen and liver.
- they are similar to alkaline phosphatases in the fact that they are concerned with the transfer of phosphate from one alcohol to other . the modern studies show that there are more than one type in paraffin section dew axed occur by benzenoid hydrocarbon and followed by acetone because alcohol cause inhibition of enzymes.

Dehydrogenases :

- They dehydrogenases are enzymes which can remove hydrogen from the substrate and transfer it to another substance the dehydrogenases which can be demonstrated histochemical are the following :
- Succinate dehydrogenases malate dehydrogenase , glutamate dehydrogenate glucose phosphate dehydrogenases …….etc.

*Principles of dehydrogenases histochemical:-

- This depend on the following Dehydrogenases remove hydrogen from the substrate the releasing hydrogen reduces the tetrazonium salt (which is colorless) forming a Formazan which is color soluble
- Formazan is chelated with cobalt to form colored insoluble granular deposit at the site of enzyme Succinic dehydrogenase
- This enzyme is an example for dehydrogenases and can be histochemical demonstrating by the following sodium succinate is used as a substrate for succinic dehydrogenase at a PH76
- The incubating medium is the Neotetrazolium salt
- The hydrogen released from the substrate by the enzyme, reduces.
- The Neotetrazolium salt forming a Formazan which is then chelated with cobalt to produce a colored insoluble granules deposit at the site of enzyme.

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