المحاضرة الرابعة

"Clinical Biochemistry"

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2008

Clinical Biochemistry :

is a chemical application to study a healthy and pathological states of human & it give information for diagnosis and treatment of the diseases & also preventing a disease in human .

The patient investigated clinicaly by testing a sample taken from any biological fluid in the human body, the important tests & common samples :

* blood and its derivatives (veins , arteries , capillaries
* Urine , e.g urea , uric acid for kidney function .
* Cerebrospinal fluid (CSF)
  test for chloride , electrolyte , protein , glucose in CSF.
* Gastric juice
* synovial fluid
* Duodenum fluid
* saliva
* feces
* sweat

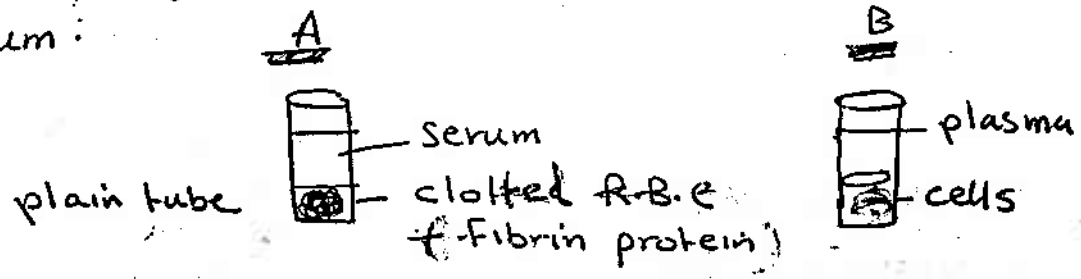
The blood is considered a basic in clinical chemistry , most of component of cells is transported by the blood . The urine test is important in the clinical chemistry .

Blood : is a suspension of cells in a protein – salt matrix . to obtain a whole blood cell add anti coagulent .

Blood

- Non cellular portion (extracellular) plasma
  - Serum
  - Fibrinogen
- Cellular portion Whole blood cell

To obtain serum, the blood is usually drawn from patients by disposable syringe & transferred to clean plain tube or centrifuge tube, left for 5–15 min at room temperature until coagulation process is complete by changing fibrinogen to fibrin protein & separate to obtain serum:



The non cellular fluid, can be separated from the clotted material, is termed serum.

The serum must be separated for a short time, from clot because the presence of clot causes hemolysis and destruction of R.B.C. This change the component of the blood & give wrong result. Also cause contamination

Sample container in clinical chemistry laboratory

This depend on the nature of sample & the period of time, because the sample must kept in container befor analysis.

Anticoagulants

Chemical substance added to the whole blood sample to obtain plasma.

Types of anticoagulants:

① **Heparin** : (Sulfated mucopolysaccharide)

(a) Formed from : (1) Glucuronic acid
(2) glucosamine
(3) acetic acid
(4) sulfuric acid

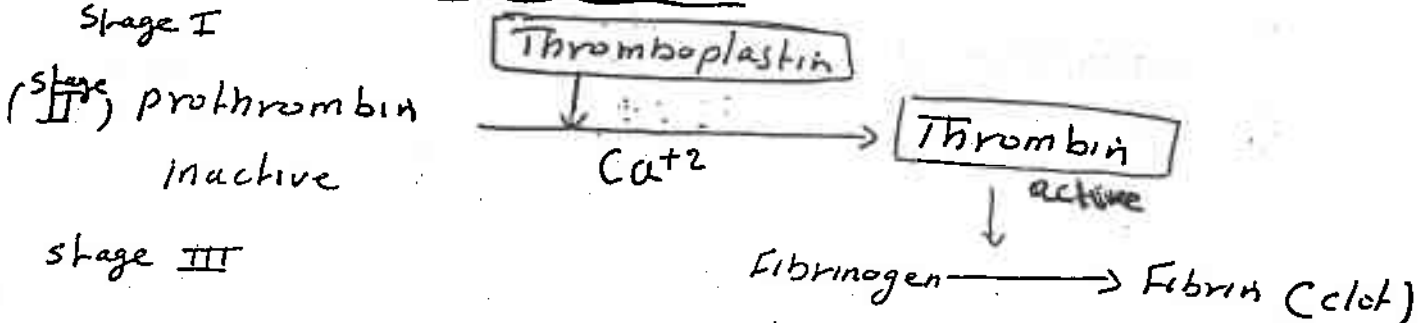
these four molecules are joined by α 1,4 glycosidic linkage.

(b) It is naturally present in blood in small concentration 0.2 mg / ml blood.

(c) The source of heparin in the body is liver, spleen, Lung.

(d) Function act as antiprothrombin and anti-thrombin (inhibitor of prothrombin)

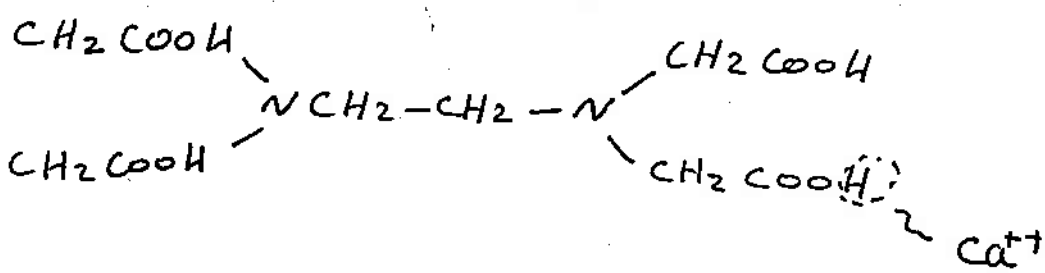
Mechanism of Coagulation



Prothrombin, calcium, Fibrinogen } are all normal constituents of the blood

Thromboplastin is not normal = of blood, but when blood vessel and tissue cells are damaged, thromboplastin is liberated from Thrombocytes.

② E.D.T.A. The compound act by chelating with Ca^{++} which is necessary for clotting mechanism.
Ethylene diamine tetraacetic acid, this compound prevent blood clotting by combining with Ca^{++} which is present in the blood & form complex compound.



③ Oxalate:
Used in tests of clinical chemistry but not useed for calcum. This compound remove Ca^{++} ion by precipitation as insoluble Ca-oxalate salt.

④ Sodium Fluoride: this type of anticoagulant is Used for glucose test, becaus fluoride ion or inhibit prevent glycolysis by inhibiting enolase enzyme; it means preventing reduction in blood glucose as results of glycolysis.

Kit: Combination of reagent suitable for limited number of samples or tests useful for research work or routine work as in small laboratory.

Separation and Techniques Used in clinical Biochemistry

Substances which differ from each other are easily separated, the separation are usually based on the followings:

1. Difference in molecular size or mass
2. Difference in solubility
3. charge of substance involved

The following table summerizing separation & purification technique used in clinical chemistry:

Techniques	property	Description
① Precipitation	Solubility	some substance ppt while other remain in the solution.
② Ultrafiltration and Dialysis	Molecular size or mass	Some substances pass through a sheet or a tube of porous material while other retained.
③ Extraction	solubility	Some substance dissolve more in water & other dissolve in organic solvent - e.g separation of drugs

④ Chromatography	Solubility	Some of the substanc dissolve in the immobile phase or solid supportin medium, while other dissolve more in the surrounding organic solvent mobile phase
⑤ Gel Filtration	molecular size or mass	Some substance diffus into the porous solid material, while other remain in the surrounding stream of flowing water
⑥ Ion exchange	electrical charge	some of the substance are bounding by immobile charge on the solid supporting media, while other are not bound
⑦ Electrophoresis	electrical charge	The substance with more charge move faster & the substance with opposite charge move in the opposite.

① Precipitation

either (a) substance to be analyzed is precipitated
(b) or interfering substance is precipitated

The precipitated substance or compound can be isolated or separated by filtration or centrifugation.

Centrifuge : instrument used to separate parts of cell by ppt at different speed.

Turbidometric : measurment of turbidity of the substance

There are a number of test interfer with protein therefore protein must be ppt by:
heat , acid , base , organic solvent , salt of metal ion.

In clinical chemistry there are 3 method of ppt of plasma protein the most common techniques for preparation of protein free filtrate (PFF) is:

① Folin - Wu Method precipitation :

Use tungstic acid (H_2WO_4) to ppt the protein , this is generated from addition of exact quantity of sodium tungstate and sulfuric acid , the solution should be neutral . The filtrate of folin Wu is very clear & suitable for many measurment

$$Na_2WO_4 + H_2SO_4 \xrightarrow[\text{medium}]{\text{neutral}} \underbrace{H_2WO_4}_{\text{tungstic acid}} + Na_2SO_4$$

very clear filtrate
Folin-Wu filtrate

This is the most common technique for preparation of protein free filtrate .

② Trichloroacetic acid (TCA) or picric acid

It precipitate protein because the group of positive charge of protein is neutralized by $CCl_3O_2^-$ which cause protein to have less charge & less able to be in solution.

③ Somogyi -Nelson Method.

(Alkaline zinc sulfates)

Aqueous solution of two salt barium hydroxide $Ba(OH)_2$ & zinc sulfate is added to certain amount of sample & ppt protein as zinc protein complex. which stick to the barium sulfate & form insoluble ppt.

This method provide very clear protein free filtrate

$ZnSO_4$ / $Ba(OH)_2$ + Sample contain protein ⟶ zinc protein complex ppt

Example of blood constituents commonly determined in protein free filtrate are; Urea, Uric acid, glucose, Creatinine & non protein nitrogen.

(2) Ultrafiltration & Dialysis

Ultrafilteration :
involve afiltration of biological fluids by using some kind of membrane which has a property of allowing only small size of molecule to provide protein free filtrate.

Dialysis :
refere to the diffusion of solute species or molecule across semi-premeable membrane as a result of different in chemical potential of solute on either side of membrane.

هذه الطريقة ستخدم لتنقية الانزيمات والبروتينات للحصول على مستخلص انزيمي نقي يتم من خلال هذه العملية التخلص من الاملاح والعوالق المرتبطة بالانزيم اضافة الى البروتينات العالية الوزن الجزيئي عن طريق النافذ الغشائي عبر اكياس الديلزة.

من امثلة الديلزة Dialysis مثل السيلوفان (cellophane) الغشاء الذي يسمح بمرور الجزيئات الصغيرة الحجم من خلال مسامات الغشاء وتحجز الجزيئات الكبيرة ذات الوزن الجزيئي المرتفع فقط

(3) Extraction :
by extraction, some component which is present in normal biological fluid can be separated also some drugs can be separated by this method.

(4) Chromatography)

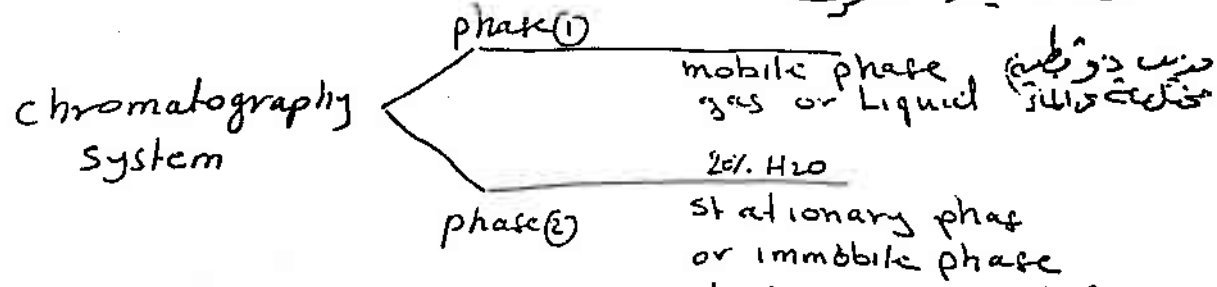
is a technique used for separating and analyzing a mixture of chemical substance and depends on the difference in the degree of interaction of each component of the mixture .e.g paper chromatography.
It consist of 2 components :

① Stationary phase or solid supporting phase (طور ثابت)
paper cellulose material provide suitable solid supporting phase which contain about 2% of water bound on the surface of paper and serve as immobile phase. This water is bound by hydrogen bound on the hydroxyl group of cellulose

② Organic solvent is used as mobile phase (طور جاري أو متحرك)
The substance to be separated will partioning between bound water & flowing organic solvent.
The more soluble compound in the flowing solvent moving faster a Long the paper, while substance which are more soluble in water move slowly or do'nt move.

Chromatography, depends on change of pH, ionic strength, biological specificity

تحليل يفصل مكونات النموذج المراد فصله تبعاً للجزئية بين الطورين الثابت والمتحرك



(Chromatography)

Uses of paper chromatography in clinical chemistry :

① For separation of polar molecule with low Mo. wt such as

(a) Separation & identification of amino acid

(b) Separation of drugs in urine & in born error disease to identify amino acid

Gel Filtration :

This method is used for separation of large molecules for examples :

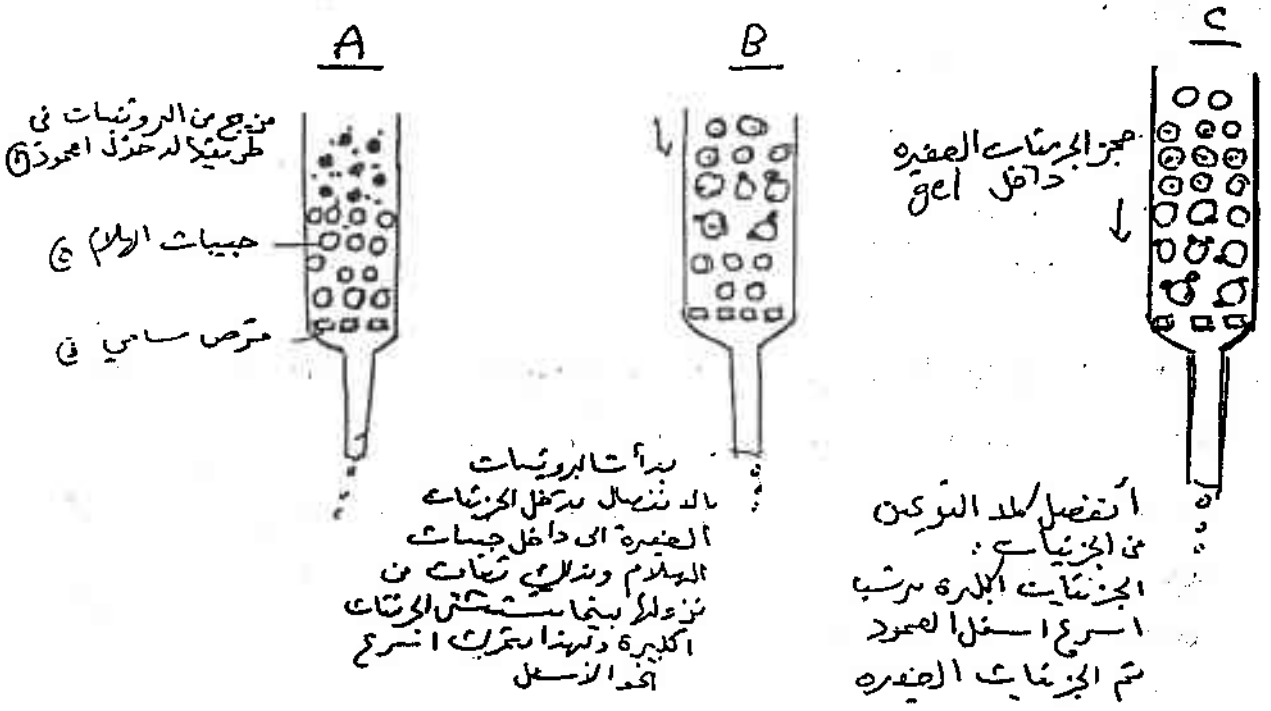
* Protein
* Carbohydrates
* Amino acid
* Ribosomes
* bacteria

It is used for estimation of molecular weight For separated molecules by using a protein with known molecular weight (standard or reference) as index to determine the molecular weight of unknown protein.

Gel Filtration : (الترشيح الهلامي)

هذه التقنية تعتمد الفصل وتنقية الانزيمات على حجم الجزيئات وتستخدم عمود مملوء بمواد (Sephades) سيفادكس وهي مشتقات سكرية متعددة أو بايوجيل (Bio - Gel) التي تعمل على تكوين الرابطة الستاهيدية بولمرية الذي هو متعدد agarose أو polyacrylamide .

حيث يملئ العمود بهذه المادة ثم يضاف محلول ممزوج من البروتينات الى اعلى العمود المملوء بهذه المادة . تبدأ عملية الترشيح بالاضافة المستمرة لمحلول منظم ذات pH معروف وبهذا فإن أكبر الجزيئات البروتينية تتحرك بسرعة كبيرة خلال العمود بينما تتشتت الجزيئات الصغيرة داخل مسامات الهلام وذات سرعة مركزة أقل



(الشكل يمثل الفصل بالترشيح الهلامي)

(6) Ion-exchange

It depend on electrostatic attraction between opposite charge, the material for ion exchange is made of cellulose or sephadex. It is used for purification of enzyme. The enzyme & materials must be in opposite charge

(7) Electrophoresis

Charged molecule in solution move in an electric field toward oppositely charged electrode is called electrophoresis, cation move to the cathode and the anion to the anode

Separation depend on net charge, molecule of different net charge move in opposite direction & those with the same net charge in the same direction but at different rate.

Separation depend on :
* Molecular structure of protein
* Ionic strength, pH
* Size of molecule

This method is suitable for separation of large molecule like plasma or serum protein, enzyme, Lipoprotein