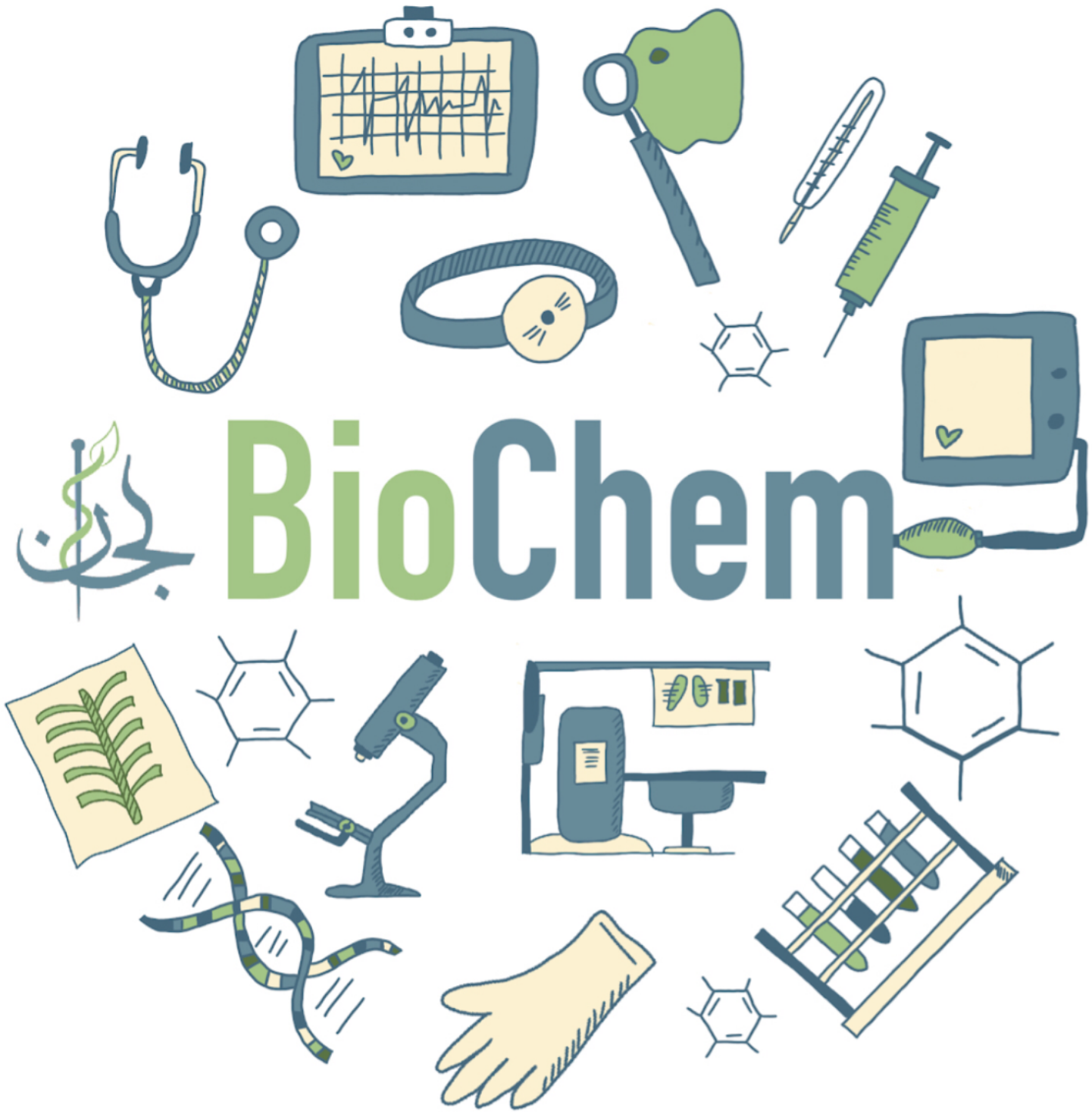

BioChem

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Enzymes in medical diagnosis:

What is the idea behind using the enzymes in medical diagnosis?
How can enzymes be used to diagnose diseases?

- If you have a blood sample and you quantify the amount of an enzyme that it has , this quantification will tell you if the owner of the blood sample has a disease or not.
- Explanation : as explained earlier isozymes existed to serve different means in different tissues that's why isozymes differ in the localisation, the idea is if you have a high concentration of a certain enzyme which is supposed to be in a certain tissue in a high concentration above normal limits in the blood this is due to the death of the tissue it serves as the cells are starting to die and opening up , all their contents are getting out which will be reflected to the extracellular space then reflected in the blood.

High concentration of a certain enzyme in the blood —> the tissue of this enzyme is **dying** —> A medical action interferes to save this tissue.

————————————————————————————————————

- **Diagnostic enzymes :**

Ex: **ALT, AST, LDH, CK(CPK)**.

-Liver disease: ALT & AST.
-Both localised is liver .

- ALT is very specific for the liver (the most specific liver enzyme) . It's a transaminase that converts pyruvate to Alanine and Alanine to pyruvate (keto acid to amino acid — amino acid to keto acid).
- AST (Aspartate transaminase) converts aspartate to oxaloacetate and oxaloacetate to aspartate. Is present in higher concentration in the liver yet it is not exclusively restricted in the liver, it is present in the heart.

- **If a blood sample reads both ALT & AST in high concentrations what does that indicate?**
- It indicates a problem in the liver.

{ if only the AST is in high concentrations it indicates a probable issue in the heart or the liver.
How to diagnose whether it's in the heart or the liver?
Symptoms, if the patient complains from chest pain —> heart.
If it's abdominal pain —> liver. }

- **AST** is more **sensitive** (higher concentration, easily read in blood) & **ALT** is more **specific** , compared to each other.

- The ratio can be diagnostic (ALT/AST).
- Less than 1 : no viral origin .
- Greater than 1: viral hepatitis, as viral cells affect the cells in a huge manner so all the tissue cells open up all together.

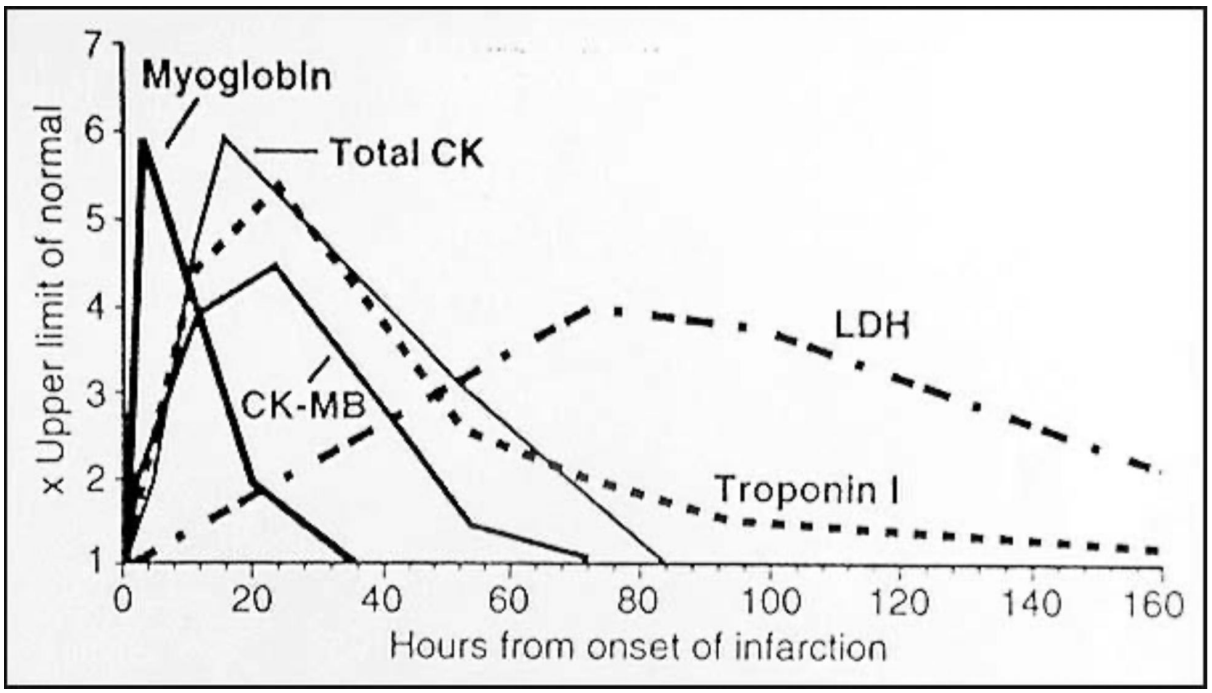
————————————————————————————————————

- **Myocardial infarction**:

-Myo —> muscle.
-cardial —> with respect to the heart.
-infarction—> the formation of a thrombi in the blood vessels - the blocking of an artery (خثرة) /(جلطة) .

1. The first step to take when a patient complains from chest pain is —> ECG ,it tells if the patient has a myocardial infarction or not. However, a normal ECG doesn't exclude myocardial infarction as a potential cause of pain.
2. Another way to diagnose a myocardial infarction is by blood tests to check out two enzymes **LDH & CK**.

To talk further about these two enzymes, first check this Graph :



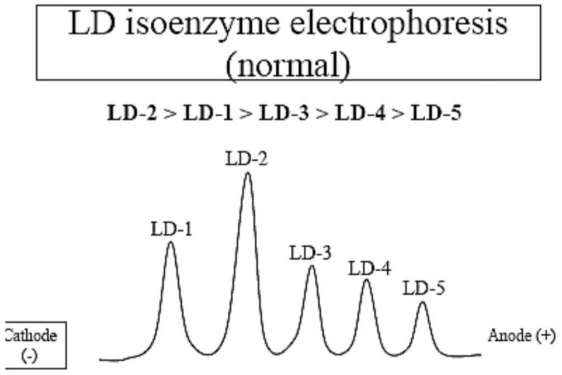
- The Y axis refers to how many folds of this enzyme are now in the blood compared to the normal level. In brief if an enzyme got 3 on the Y axis it means currently this enzyme has its concentration 3 times higher than the normal limit.
- The X axis indicates the time starting from the first sight of symptoms, meaning the patient did undergo the infarction.

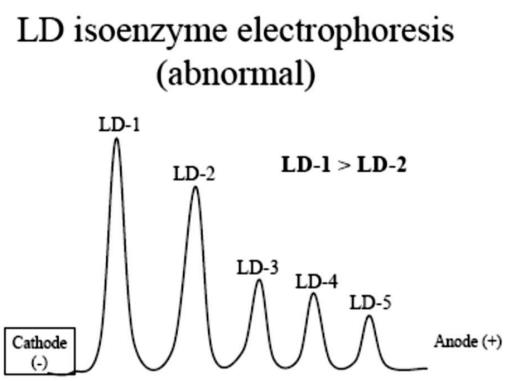
LDH:

-LDH gets its peak to 4 folds of the normal concentration approximately after three days around 70 hours. So if a patient complains of chest pain, within the first 3 days LDH is the one to check to diagnose an infarction. However, after the third day LDH concentration starts to decrease as a result LDH won't help much in the diagnosis .

- The increase of LDH generally in the blood in a myocardial infarction is due to the increase of LDH1 (already explained in sheet 29).

This graph represents both LDH1&LDH2 concentrations in two conditions:





- LDH1 has a lower concentration than LDH2 in the blood so by running gel electrophoresis in a normal condition LDH1 will show up in a lesser degree than the LDH2.HOWEVER, in myocardial infarction this ratio will be flipped LDH1 concentration will be higher than LDH2 concentration .
- LDH-1/LDH-2 ratio is diagnostic for myocardial infarction (heart attacks).
- Normally, this ratio is less than 1.
- Following an acute myocardial infarct, the LDH ratio will be more than 1.

CK:

-It's a dimer formed of two subunits and has three isozymes.
-The two subunits are : M subunit & B subunit.

- B —> represents the Brain.
- M —> represents the skeletal muscles.

-A two B subunits Ck enzyme is localised in the brain & A two M subunits Ck enzyme is localised in the skeletal muscles.
-if a patient complains of unexplainable headaches and when you run blood tests for a specific isozyme & the test shows that Ck the bb type is higher in concentration than normal, as a result you are up to diagnose the patient with a potential brain cancer.

- if the Ck mm type is higher in concentration than normal you are expected to have a skeletal muscle tumor or cancer or maybe an injury to the skeletal muscles.

This graph indicates the location of these isozymes:

Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB **20% MB** **80% MM**	97% BB 3% MB 0%MM

- Heart, skeletal muscles, & brain.
- Like LDH, there are tissue-specific isoenzymes of CPK:

- CPK3 (CPK-MM): the predominant isozyme in muscle.
- CPK2 (CPK-MB): accounts for ≈35% of CPK activity in cardiac muscle, but less than 5% in skeletal muscle.
- CPK1 (CPK-BB) is the characteristic isozyme in the brain and is significant in smooth muscle.

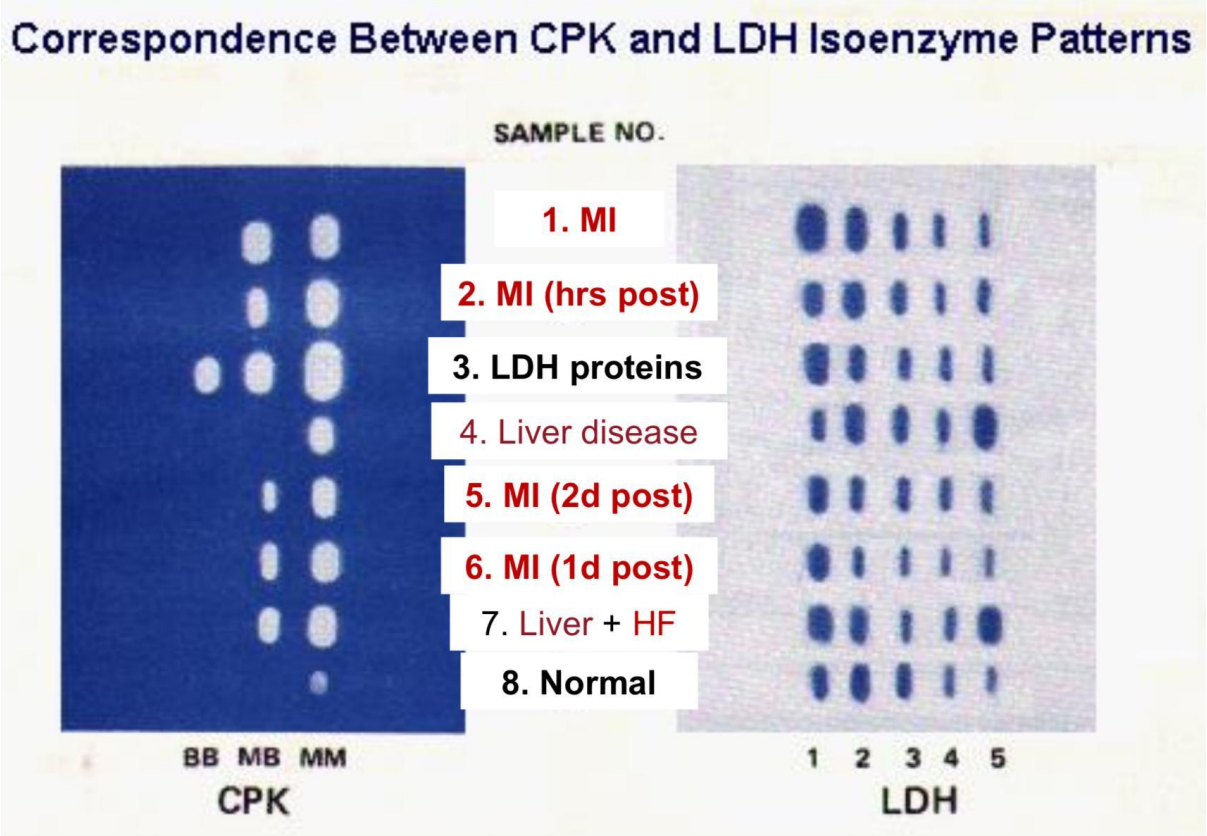
[The increase in the total CK concentration is primarily due to the increase of CK-MB, so total CK reaches around 6 folds of the normal level & CK-MB is responsible for 4.5 folds in increase]

- This enzyme reaches its peak in the first day of infarction after 24 hours the level decreases losing its diagnostic importance.

If you run a test for CK-MB and it's higher than the normal what would it be?

- It would be another infarction as it's common for patients with infarctions.

This graph shows a comparison between LDH & CK :



+ In gel electrophoresis, when bands get bigger it means a higher concentration of a specific protein & smaller ones are with lower concentration.
+ When two bands are next to each other yet one of them is bigger this means that the ratio is greater than 1 and so on .

Explanation for the 8 samples :

Sample #3 represents results for a control.
Sample #8 results are from a normal specimen.
Sample# 1 MI patient. The specimen was collected at a time when the activity of both LDH and CK were
elevated. Note the LDH flip and the high relative activity of the MB isoenzyme.
Sample# 2 MI patients who experienced chest pain only several hours previously. Total CK is significantly
elevated with a high relative MB isoenzyme activity.

Sample# 6 MI patient (the 1st day post MI); CK activity is definitely elevated with a high relative MB
isoenzyme activity and the LDH flip is evident.

Sample# 5 MI patient (2 days post MI) so that CK has almost returned to normal activity and the LDH flip
is definite.

Sample# 7 MI patient with complications of heart failure and passive liver congestion or the patient was
involved in an accident as a consequence of the MI, and suffered a crushing muscle injury.

Sample# 4 a patient with liver disease. Although the LDH isoenzyme pattern is indistinguishable from
muscle disease or injury, the absence of at least a trace of CK-MB isoenzyme is inconsistent with the muscle CPK isoenzyme distribution as is the apparently normal total activity.

Protein Purification and Characterization Techniques

Extracting Pure Proteins from Cells:
Many different proteins exist in a single cell. A detailed study of the properties of any one protein requires a homogeneous sample consisting of only one kind of molecule. The separation and isolation, or purification, of proteins constitutes an essential first step to further experimentation.

-Purification techniques focus on size and charge mainly.

Many techniques are performed to eliminate contaminants and to arrive at a pure sample of the protein of interest.

How do we get the proteins out of the cells?

Before the real purification steps can begin, the protein must be released from the cells and subcellular organelles.

- The first step, called ***homogenization***, involves breaking open the cells … BY :

1) The simplest approach is **grinding ,** it has been used in the past by **Potter–Elvehjem** homogenizer {in the figure }.

2) **Sonication (like ultrasounds)**, involves using sound waves to break open the cells (it is being used in the medical field not to break up the cells) but increasing the frequency of these waves to a higher level, it will break up the cells.

3) Cells can also be ruptured by cycles of **freezing and thawing.**

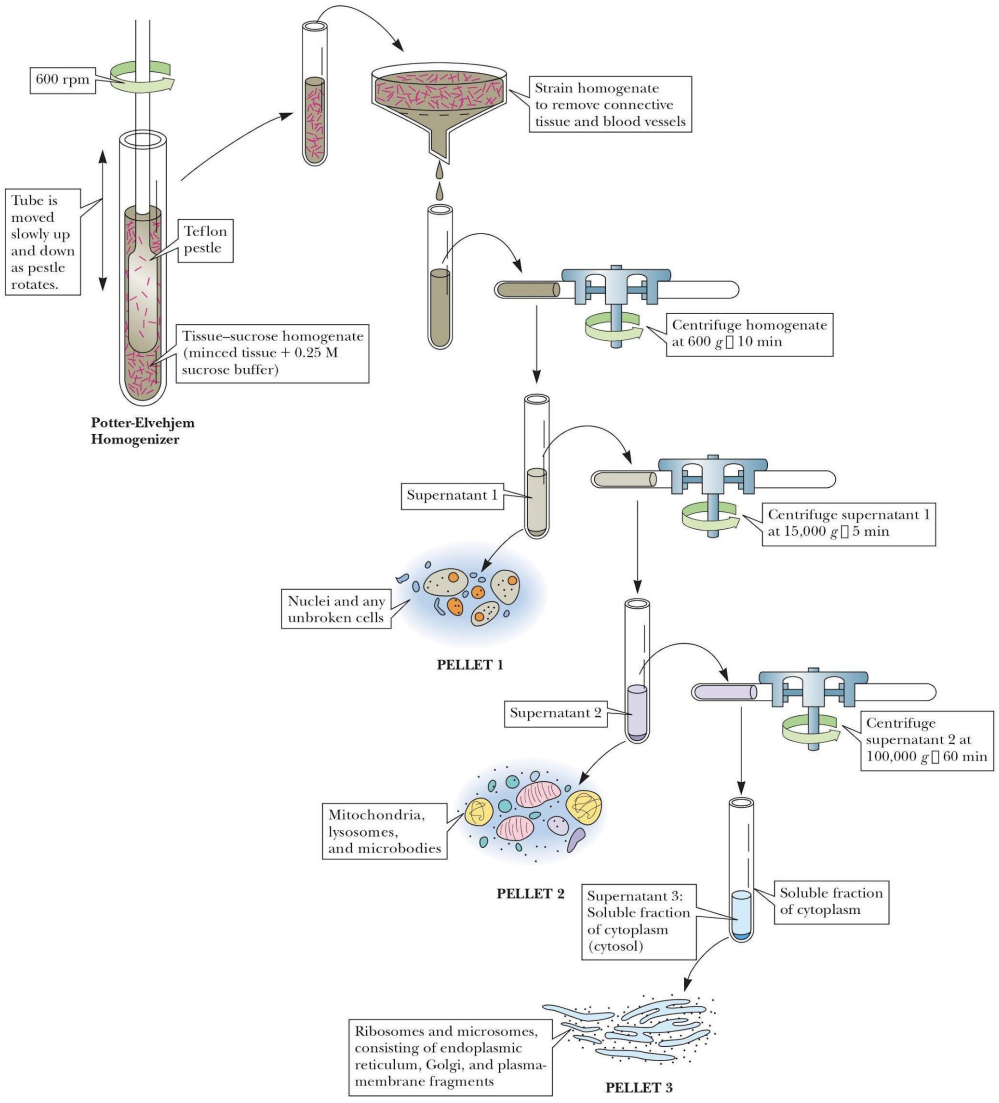
- The second step, after the cells are homogenized, they are subjected to **differential centrifugation.**

Explanation ~ [just to understand the process]

Spinning the sample at 600 times the force of gravity (600*g) results in a pellet of unbroken cells and nuclei.

If the protein of interest is not found in the nuclei, this precipitate is discarded. The supernatant can then be centrifuged at higher speed, such as 15,000 *g, to bring down the mitochondria. Further centrifugation at 100,000 * g brings down the microsomal fraction, consisting of ribosomes and membrane fragments. If the protein of interest is soluble, the supernatant from this spin will be collected and will already be partially purified because the nuclei and mitochondria will have been removed.

*this figure is from the book, for clarification



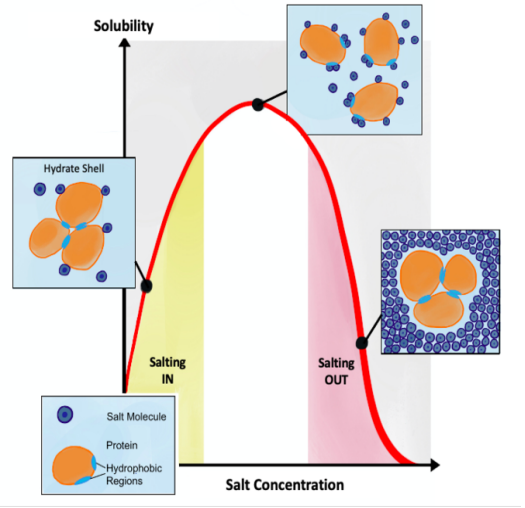
Salting in & out

- Are proteins soluble? If yes, to which limit?

Proteins have varying solubilities in polar and ionic compounds, but what will happen if we add salt to the protein solution?

Salting in

Proteins remain soluble because of their interactions with water. When salt is added to a protein solution ,Salt stabilizes the various charged groups on a protein molecule and enhances the polarity of water and increases the ionic strength, thus attracting protein into the solution and enhancing the solubility of protein.



Salting out

If we add more and more salt , some of the water is taken away from the protein . With less water available to hydrate the proteins, so decreasing of proteins solubility , they begin to interact with each other through hydrophobic bonds.

At a defined amount of salt, [FIRST] a precipitate that contains contaminating proteins forms. These proteins are the ones with a lower solubility . Then more salt is added, and a different set of proteins precipitates. This precipitate is collected and saved.

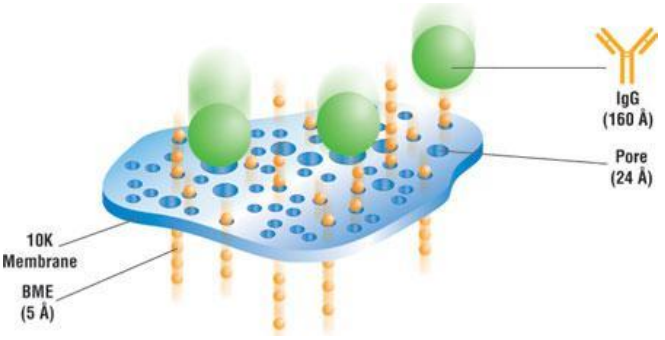
*Ammonium sulfate is the most common reagent to use at this step

*This technique is important but results are crude ….. but cheap , simple, and convenient .

Dialysis

This process is depending on Principle of diffusion , and Molecular weight. (MW cut-off)

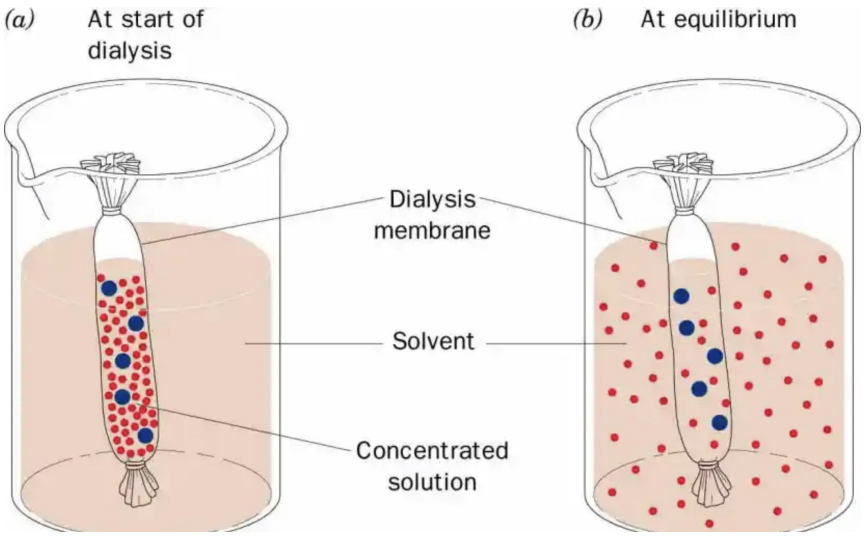
1. In dialysis a semipermeable membrane is used to separate small molecules and proteins based upon their size (depending on MW).



2. A dialysis bag made of a semipermeable membrane, and has small pores.
3. The bag is filled with a concentrated solution containing proteins. Molecules that are small enough to pass through the pores of the membrane diffuse out of the bag into the buffer/water solution.

-results of this technique is crude , because any protein which has a MW above that cut (MW cut-off) is contained inside the membrane .

*cheap , simple, and convenient .



Column Chromatography

The word chromatography comes from the Greek **chroma**, “color,” and **graphein**, “to write”.

We have a sample that contains a colored protein, the idea ;

1. We have a material which has the ability to bind your protein of interest and then to have that material inside a column and
2. you will pull your sample which has your protein of interest over the column, now the sample will start to move inside the column and when the sample gets out (colored).
3. You will see the color of interest(protein of interest) start to get out ,collect it inside beakers .

- This column is connected to a pencil and it will write on a drum the intensity of the peaks and the wavelengths ,so the higher the concentration(of proteins of the sample), the higher the peaks.

- This technique depends on two concepts (phases) :

The **stationary phase**, and the other is the **mobile phase**. The mobile phase flows over the stationary material and carries the sample to be separated along with it. The components of the sample interact with the stationary phase to different extents. " Some components interact relatively strongly with the stationary phase and are therefore carried along more slowly by the mobile phase than are those that interact less strongly or Some components bind to the stationary phase . The differing mobilities of the components are the basis of the separation ."

e.g (positively charged stationary phase and the protein of interest is negatively charged: protein of interest will bind to the positively charged stationary phase, and the positively charged proteins won't bind, they will get out)

- Washing: means getting materials (which aren't bound to the stationary phase) outside the column .
- Elution: means getting materials (which are bound to the stationary phase and contains the protein of interest) outside of the column.