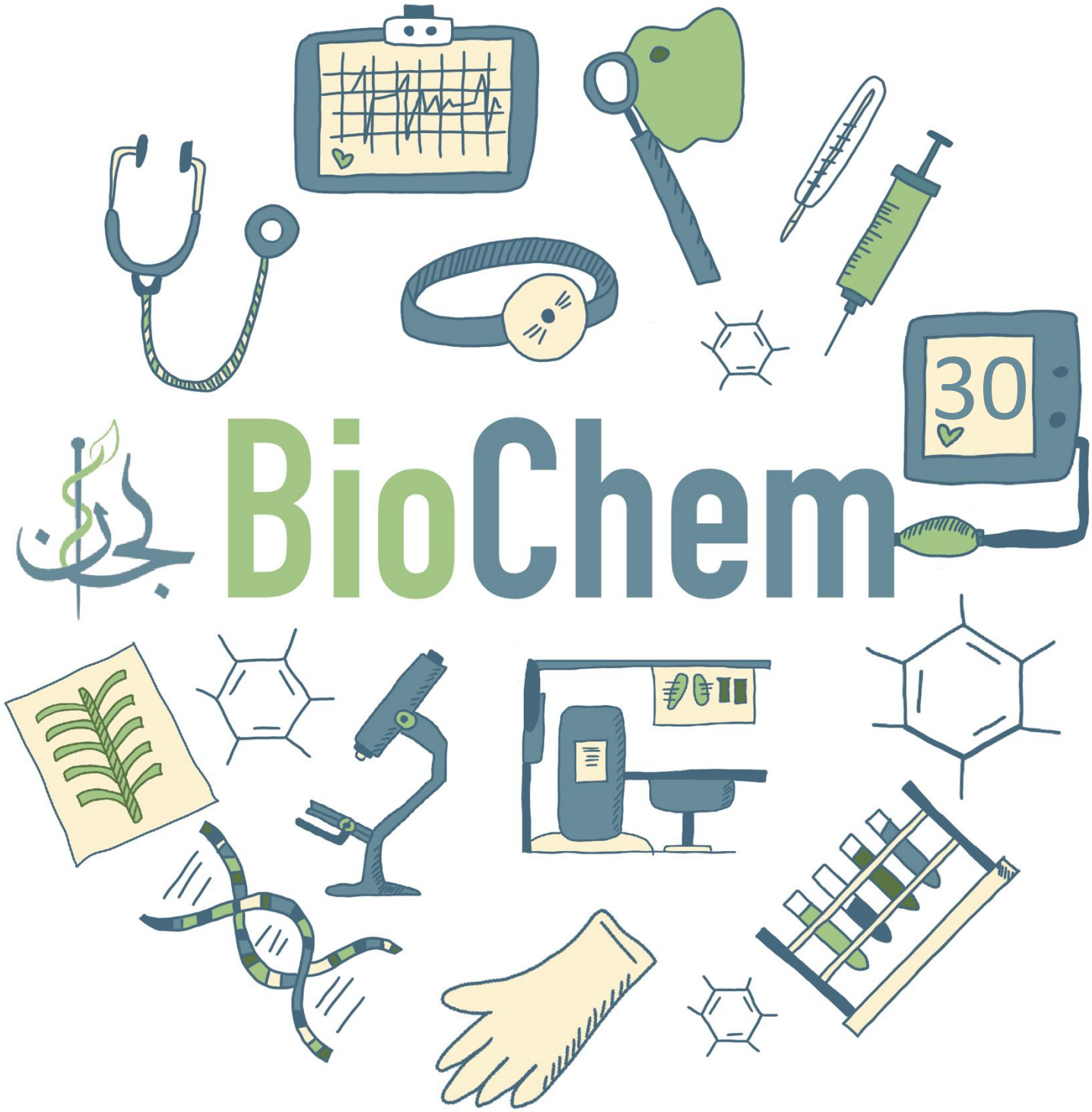

BioChem

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Abzymes

Abzymes: are **antibodies** that have catalytic activity and **are produced against a transition state analog**.

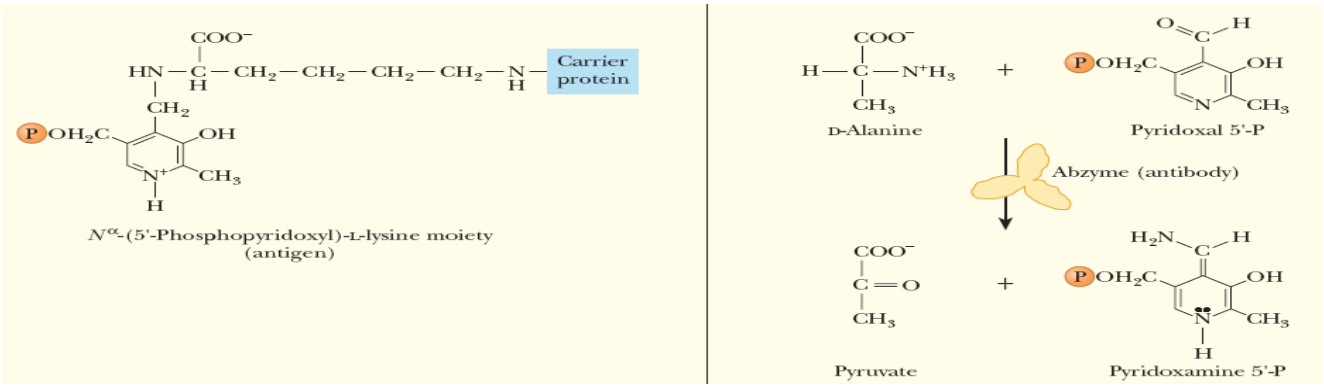
➔ We said before that we can't synthesise a transition state (a substrate in a transition state), but the idea here that we synthesise a material which looks like transition state (analog) and inject it in **an animal**, so the animal will synthesise antibodies against it (because its foreign).

➔ As we know that antibodies are proteins and the molecule is like a transition state, so the binding between them will looks like the active site.

*then we extract the antibodies from the animal to use them, and now antibodies have a catalytic activity (they don't have it normally) because they were synthesised against molecules which look like the transition state, and it will have higher affinity of binding to substrate (because its very specific) than enzyme.

-We call these catalytic antibodies -> Abzymes.

=remember that it produced in animals.



ملاحظة

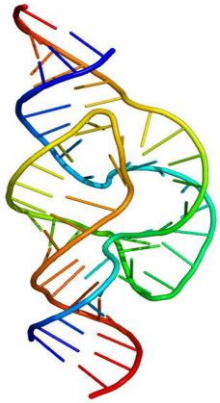
تستخدم هذه الطريقة لمعالجة المدمنين, بحيث يتم حقن المريض بهذه ال abzymes والتي تقوم بتكسير الكوكائين لجزيئين غير ضارين لا يسببوا الإدمان فيقل تركيز الكوكائين في جسم المريض.

Ribozymes

e.g. on ribozymes: 1. **telomerase** and 2. **RNase P**

Ribozymes are the exception of the enzymes (they are not proteins they are **RNA molecules** that has properties of catalyzing chemical reactions.

- ➔ They catalyse reactions that have relationship to the RNA itself (i.e. Catalyse splicing reactions and are involved in protein synthesis).
- ➔ The catalytic efficiency of catalytic RNAs is much less than that of protein enzymes, it can be greatly enhanced by binding to specific protein, even though it still less than protein enzymes.



Regulation of Metabolic pathways

=Reactions in the body always exist as a series of reactions (as when glucose is converted to pyruvate, it takes 10 steps) and rarely occur as single reaction, the series of reactions are called **pathways**.

Pathways (The series of reactions) can have many types:

1} **Linear pathway**: In this pathway each material leads to another material until we reach the final product and each step is catalysed by specific enzyme different than each other.

=> example: Glycolysis (which we take in bio 101).

2} **Cyclic pathway**: same in concept to linear pathway but here the final product is same as the first molecule that we start with (actually it's the first molecule itself).

=> example: Kreps cycle (citric acid cycle or TCA cycle, the same), urea cycle.

3} **Spiral pathway**: also the same concept with the linear but here each step is catalysed by the same set of enzymes.

Principles of pathway regulation

Because the reaction has many steps you should know specifically where to control, for example, regulation of the first reaction (generally and usually very high regulation in this step, on the other hand the last step there

will be the least regulation), regulation of first step will make you avoid the synthesis of different intermediates, or if you have more than one way to complete the reaction you can regulate to reach what you want.

The regulation in our bodies follows these principles:

1] **Counterregulation of opposing pathways**: in our body the synthesising reactions occur in specific place away from degradation reactions.

=> For example, all degradation reactions occur in the mitochondria (except for the glycolysis), and all synthesising reactions occur in the cytosol, so they don't occur in the same place.

2] **Tissue Isozymes for regulatory protein**: by putting every isozyme in different places making the reaction behave differently in different tissues.

> **Remember**
> Isozymes:
> are enzymes that differ in amino acid sequence but catalyze the same chemical reactions.

3] **Regulation at the rate-limiting step**:

* the rate determining step is the slowest step, it usually not reversible and it's the first committed step (we'll discuss it in a while).

=> Pathways are principally regulated at their rate-limiting step because Changes in this step can influence flux through the rest of the pathway.

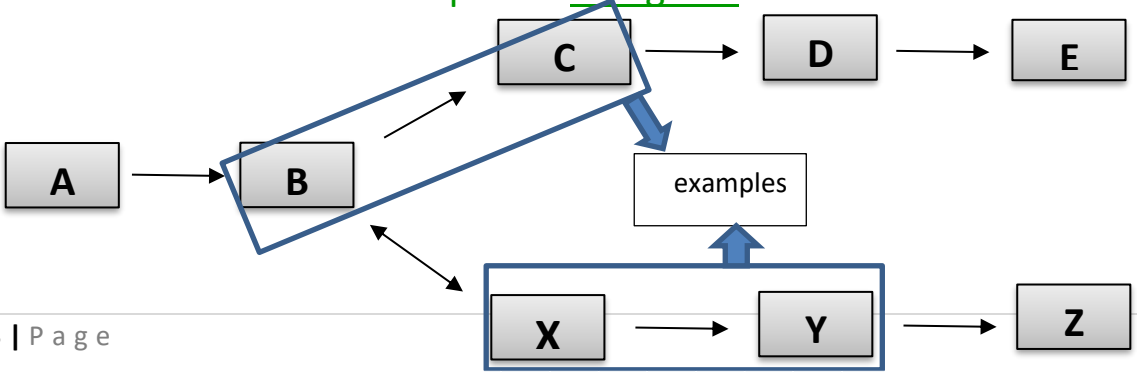
➔ our body realise the rate-determining step by its properties:

1} it requires high amount of energy (has high activation energy).

2} it has high Km values which mean that it needs high concentration of substrates.

4] **The committed step**: a committed step in a metabolic pathway is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return.

*note: the committed step is an exergonic reaction.



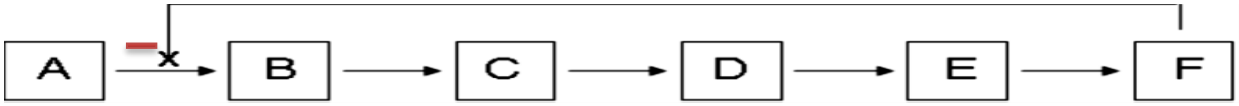
= the committed step for making E is (B → C), while the committed step for making Z is (X → Y) not (B → X) because its reversible (لاحظ السهم المنعكس).

5] **Feedback Regulation**: to modify or behave according to what you got.

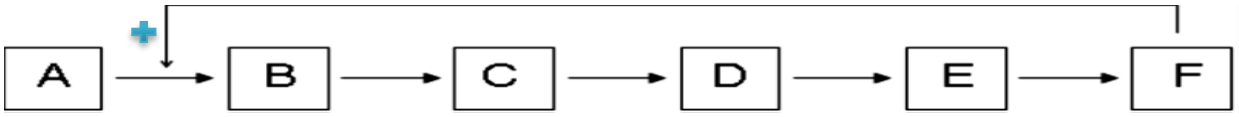
-> This type of regulation is much slower to respond to changing conditions than allosteric regulation.

Types of feedback regulation:

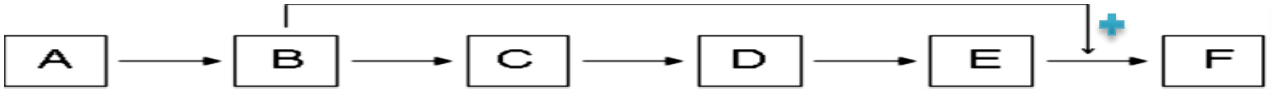
A) Negative feedback regulation: If the final material is increased to a certain level it will come back and inhibits the first enzyme that catalyses the whole pathway.



B) Positive feedback regulation: If the concentration of the final material increased to a certain level it will come back and give more activation to the enzyme that catalyses the first step (the body use it when it needs more and more of the product).



C) Feed- forward regulation: when the first intermediate comes out it goes to a step ahead of it and catalyses the enzyme ahead to it to be more active, so whenever the intermediate comes, it will be consumed directly (the body use it when it wants to get red of toxic intermediates in the liver by increasing the prosses of consuming these toxic materials into nontoxic products).



6] **Enzyme compartmentalization**: this concept means that the enzymes that are needed for a certain process are put in a certain place of the cell with there substrates as the amount of the enzymes and the substrates are relatively low, and that's why we have organelles within the body.

→ the idea is instead of leaving the substrate searches for the enzyme in the whole cell, we but them together in a certain part to fasten the reaction to occur and reducing the area of diffusion, for example:

-we find the hydrolytic enzymes in the lysosomes.

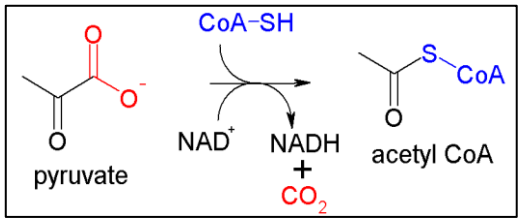
-we find all the energy metabolic pathways in the mitochondria.

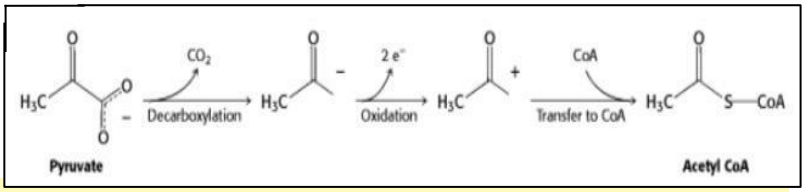
-synthesis of fatty acids occur in the cytosol, while their degradation occurs in the mitochondria.

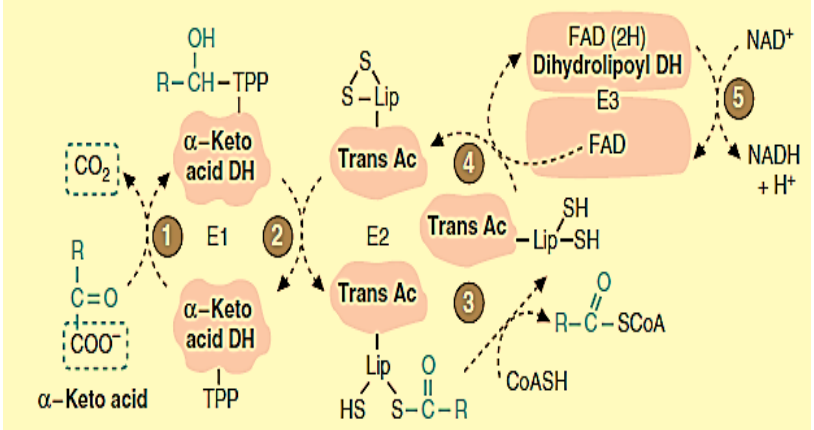
**In this way, enzymes are sequestered (معزول)inside compartments where access to their substrates is limited.

7] **Enzyme complexing (a multienzyme complex)**: some enzymes have the term 'complex' at the end of its name, as (α-ketoglutarate dehydrogenase complex) and this term means that we are having more than one enzyme complexed together (bounded together) each one does a certain function different from the others, but the idea is that the product of first enzyme isn't used except by the second one so we find these enzymes connected to each other and the process repeats (this will help by reducing the time and the energy needed to make the substrate reaches the enzyme, for example:
-pyruvate dehydrogenase: a prosses catalysed by 3 enzymes and have 3 different processes (pictures are important):

1. decarboxylation, removing the COO^-.
2. oxidation of the first product by reducing NAD+ to NADH.
3. transfer of the resultant acyl group to CoA.







*About this figure:
(1) the first enzyme is decarboxylase, which remove COO^- and remember that it uses TPP coenzyme (2).

3 the rest carbon will be loaded in the second enzyme which is trans acylase, here CoA will bind the carbon and it leaves the reaction as acetyl CoA.

4 Oxidation occur by the third enzyme which transfer the $\mathbf{e}^-$ to FAD to become FADH2 and then 5 NAD^+ come and take the $\mathbf{e}^-$ and becomes NADH.

=> these are three different enzymatic reactions that occur in the same time.

Enzymes in Medical Diagnosis

- Enzymes are not evenly distributed in our bodies, and that's stands behind the difference between our cell types.

➔ The reason for that is the degree of transcription and translation of the DNA and the RNA, so they differ in the protein content and as a result in enzyme content.

- Idea: if certain enzymes are found in certain cells, and these cells died and disintegrated (تفككت), these enzymes will diffuse to the ECM then to the blood, so if we take a blood sample, we will find that these enzymes are present in the blood in higher concentration than normal, and in this way we can know that there's a problem in the cells that contain these enzymes (clinical e.g. إذا واحد راح عالطّوارئ بشكي من وجع في قلبه، عطول بعملوله فحص تخطيط القلب + باخذوا عيّنة من دمه ليشوفوا إنزيمات القلب)

Diagnostic enzymes & Liver disease

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

ALT: alanine trans-aminase enzyme

AST: aspartate trans-aminase enzyme

=For liver disease: ALT (which is the most specific enzyme for the liver disease) and AST (found in the liver and in the heart so it's less specific although it's found in higher concentration, because it's not found only in the liver but also in the heart).

=LDH (lactate dehydrogenase): It's found in the heart and in the blood and in the skeletal muscle.

فحص تخطيط القلب: ECG

*) إذا ECG غير طبيعي فهذا دلالة على وجود جلطة، لكن إذا كان طبيعي فهذا لا يعني السّلامة من الجلطة بالضّرورة ،عشان هيك بنشوف إنزيمات القلب.

=CK (creatine kinase or creatine phosphokinase CPK, the same): It's found in muscles, heart, and the brain.

For examination of the liver disease we test the concentration of ALT &AST, and then we take the ratio between them (ALT/ AST).

- ➔ In the case of liver disease or damage (but not viral origin because viruses have high effect on cells): the ratio will be less than 1.
- ➔ In the case of viral hepatitis (التهاب الكبد الوبائي): the ratio will be higher than 1.

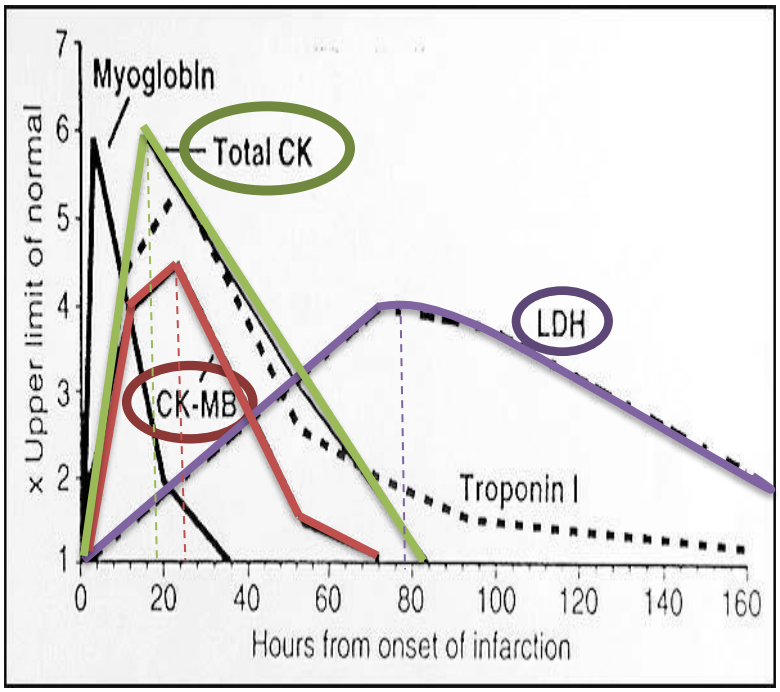
*the figure represents myocardial infraction (MI)

(جلطة قلبية):

X-axis: the time from the occurrence of the infraction.

Y-axis: 1 is the normal, and 2,3… are increasing folds.

*Here we are interested in CK and LDH.



=> CK: in less than a day it reaches its peak which is 6 folds of the normal level, and approximately in 3 days it will come back to the normal level.

NOTE: we write total CK because it has many isozymes, the isozyme which presents in the heart is CK-MB which also reaches its peak in less than one day (4 folds) (notice the red curve).

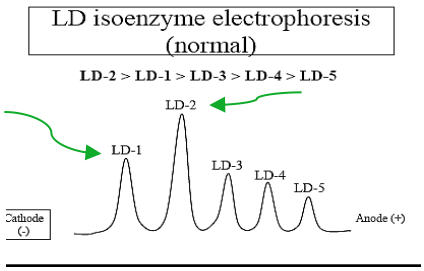
=>LDH: it reaches its peak which is 4 folds of the normal level, in approximately 3 days, and it returns to the normal within a week.

مثال: إذا اجى مريض واشتكى من ألم في الصدر اله3 أيام, وانت عملتله فحص لل CK-MBوطلع عند قمته (أربع أضعاف الطبيعي) هون لازم تعرف انه صابته جلطة ثانية (لأنو الأصل بعد ثلاث أيام يكون رجع

للطبيعي) بس بما انه عند قمته فهذا بدل على انه صار معه جلطة ثانية, وهذا الاشي شائع عند مرضى الجلطات القلبية, بتيجي اول اشي جلطة خفيفة وبترجع بعدين بتيجيه واحدة ثانية.

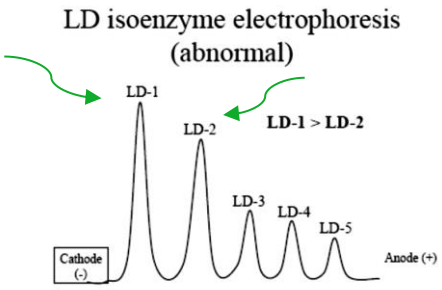
مثال 2: انو يجي مريض بعد ثلاث أيام من الم الصدر وتعمله الفحص ويكون مستواه طبيعي ف تروّح المريض (وهذا غلط شائع بين الأطباء في هذه الحالة) لانو الأصل بعد يومين يرجع لمستواه الطبيعي, فهون لازم توخذ بعين الاعتبار المقياس الثاني LDH والي لازم يكون مستواه عالي عشان يثبت التشخيص.

LDH in myocardial diagnosis: LDH has 2 isozymes, LDH-1 in the heart and LDH-2 in the RBC.



**Normally, the ratio (LDH-1/ LDH-2) is less than 1.

**in the case of myocardial infraction, the ratio will be more than 1 (diagnostic ratio).



CPK (creatine phosphokinase) (or CK):

It has 3 copies:

1} CPK_1: (CPK-BB), it founds in the brain and found in significant amounts in smooth muscles.

2} CPK_2: (CPK-MB), it founds in the heart (cardiac muscle), it counts for %35 of CPK activity in it & %5 in skeletal muscles.

3} CPK_3: (CPK-MM), it's the predominant isozyme in the muscles.

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

لاحظ أن MB متواجد في العضلات القلبية بنسبة كبيرة نسبياً لوجوده في الدماغ والعضلات الهيكلية, لذلك عندما نجد تركيزه مرتفعاً في الدم, فوراً نعرف أنه جاء من العضلات القلبية.

لم نستخدم MM لأنه متواجد بنسبة كبيرة في القلب والعضلات الهيكلية, فهو ليس دقيقاً للتمييز بينهم.

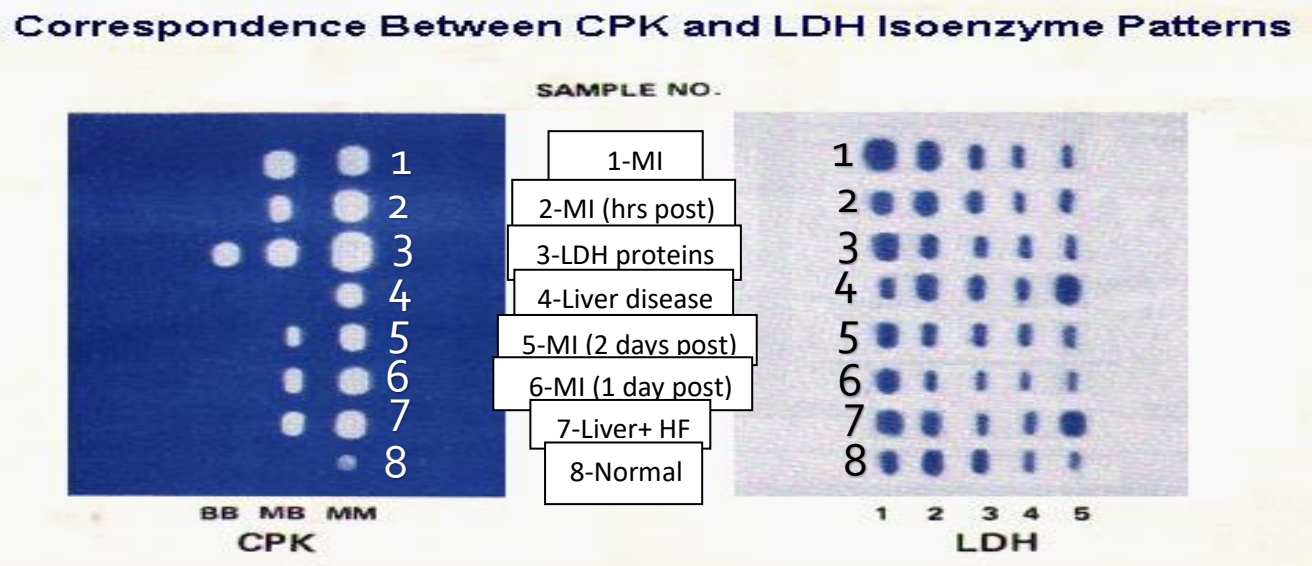
نستخدم BB في فحص الدماغ, فعندما يرتفع مستواه في الدم, فإن ذلك يشر إلى تلف في خلايا الدماغ, أي سرطان.

بشكل عام يصعب فحص كل نوع على حده, بل يتم فحصها معاً (أي أننا نفحصCPK كاملاً وليس أنواعه الفرعية) ونستطيع التنبؤ بمصدرها من الأعراض عند المريض ومكان الألم (مثلا: ألم في الرأس-مشكلة في الدماغ/ ألم في الصدر-مشكلة في القلب.. وهكذا).

**NOTES:

Most of released CPK after MI (الجلطة القلبية) is CPK-MB.
Increased ratio of CPK-MB/total CPK may diagnose acute (حاد)infarction, but an increase of total CPK in itself may not.

The figure down shows gel electrophoresis for 8 samples:
(Read the figure from below!).
*details are not important, just try to analyse the cases!
=>Sample #8 results are from a normal specimen.
=>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# 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# 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.
=>Sample #3 represents results for a control.
=>Sample# 2 MI patient who experienced chest pain only several hours previously. Total CK is significantly elevated with a high relative MB isoenzyme activity.
=>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.



Protein Purification and characterization techniques

Extracting Pure Proteins from cells

This is a method that is used to separate the proteins (or enzymes) from other cell components.

=>Purification techniques focus mainly on size & charge.

The first method is called Homogenization: here we crush the cells and its component to convert them to homogenous solution (with the liquid around the calls).

=>There are many ways to complete the homogenization:

1) Grinding, by Potter–Elvejhem homogenizer
(استخدمت قديماً وهي عملية دق وطحن للأنسجة)

2) Sonication: (sonic means صوتي (ما الها علاقة بالسرعة)): here we use sound waves, (because strong waves break the cells) (remember the effect of sound waves on the eardrum), this method is accomplished by a device called sonicator.

**معلومة: جهاز (الألترا ساوند) المستخدم في فحص الحامل يستخدم المبدأ نفسه, يعمل الجهاز على صناعة تذبذبات في الخلايا وهذه الذبذبات يختلف تأثيرها على الخلايا حسب نوع الخلية بسبب اختلاف مكونات الخلايا فتظهر تفاصيل الجنين (وجهه /أنفه/يديه...إلخ)

3) freezing and thawing (التجميد والتذويب): we know that the size of water increases by freezing, so by repeating of freezing and thawing the cell membranes break down.

4) detergents.

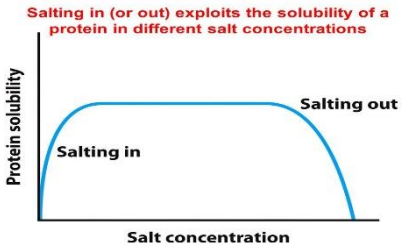
The second step is Differential centrifugation: in this prosses you can determine which component precipitate and which not by determining the speed because each component precipitate at certain speed (600 *g:* unbroken cells & nuclei; 15,000 *g:* mitochondria; 100,000 *g:* ribosomes and membrane fragments) ((g: gravity force)).

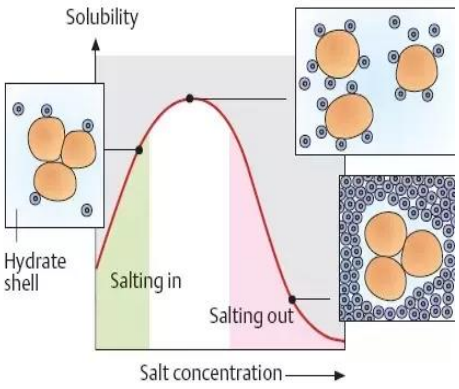
now after I had homogeneous solution, there are two methods to extract pure proteins from it:

1} Salting in & out:

=> Salts always have higher solubility in water than any other molecules (they completely dissociate in water).

=> the idea here is that when we start to add salts, its molecules start to bind water molecules, and because Na has positive charge and Cl has negative charge, when it binds to water, it makes its charge more evidence in the solution (O is – and H is +), these strong charges will make the protein dissolve better in water,, but by adding more and more salts, they will bind to water (form hydration shells) and leave small amount of water to the proteins decreasing their solubility, then the protein molecules start doing hydrophobic interactions (by their hydrophobic parts) with each other and precipitate.





**NOTES:

- When the solubility of the protein in water increased -> it takes more time to be extracted as precipitate, and vice versa.
- The results of this technique are **Crude** (not pure) because there are similarities in some proteins solubilities, so it can't be used alone to extract pure protein, but instead it used as a first step (because it's cheap) with another techniques.
- Ammonium sulfate [$(NH_4)_2SO_4$] is the most common reagent to use at this step.

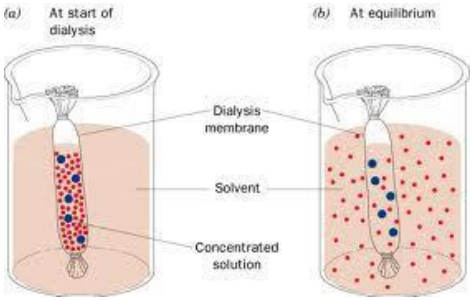
2} Dialysis:

=> the idea here is that you put the homogeneous solution which has the protein of interest in a membrane (or sack كيس) and close it from its ends by clippers (ملاقط), this membrane has pores that allow for certain material to penetrate (specific molecular weights) and then we put it in a beaker(دورق) and we put inside this beaker water or certain buffer,, at the bottom there's something like magnet which make magnetic field and start to rotate, now according to the simple diffusion, proteins (which can penetrate) start to exit the membrane.

➔ So, you should choose a membrane which doesn't allow your protein to get out and let other materials that has lower MW to get out.

The results of this technique are Crude (not pure) because of the presence of proteins with higher MW, so it also can be used before other techniques.

➔ It's also cheap prosses.



** اللون الأسود هو شرح الدكتور...
** اللون الأخضر هو كلام السلايدات...
** بالتوفيق

لا تفتر وأنت الذي اعتاد العالم على وقوفك