



Biochemistry

Title = Enzyme/3

Lec no = 3

Done By = Baraa Safi

وَقُلْ رَبِّ زِدْنِي عِلْمًا

Enzymes III

Dr. Ahmed Salem

Overview

(انزيم بيتغل نفس العمل بحول (S) الى (P) لكنة الا ختلاف في خصا ئه معينه
يعني ممكن مختلفوا في v_{max} / K_m)

- Isozymes - a group of enzymes that catalyze the same reaction but have different enzyme forms and catalytic efficiencies.
- Application of isozymes in diagnosis

Isozymes (Isoenzymes)

- **Physically distinct forms of the same enzyme**

- Multiple molecular forms of an enzyme are described as isozymes or iso-enzymes; enzymes that catalyze the same reaction
- Different molecular forms of the same enzyme synthesized from various tissues
- Useful to understand diseases

(diagnosis)

يعني ممكن ان نديم ينفرز منه القلب وينفرز نفسه الا نديم
من العضلات مثلا

- Homomultimer protein: subunits are all the same, represented by a single gene

- Heteromultimer protein: subunits are different, produced by different genes

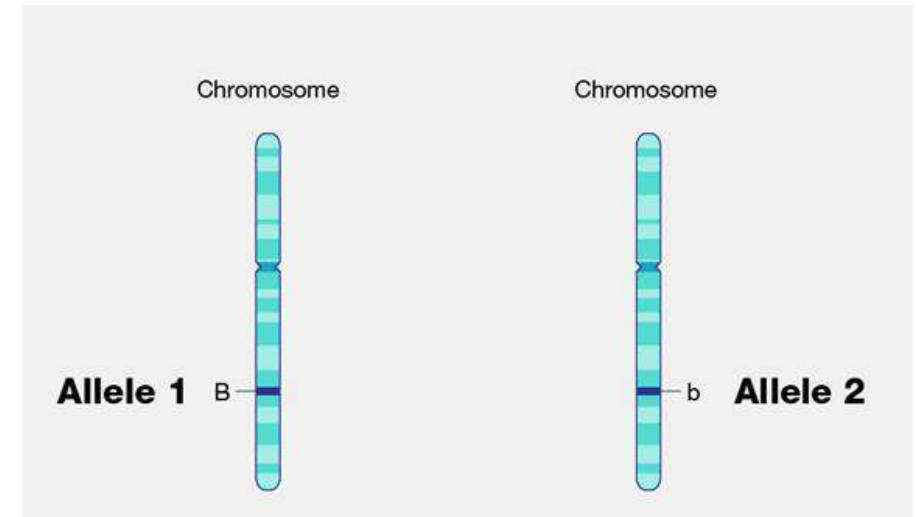
بصيروا بـ G. structure

تكرار الجين هو المسؤول عنه انتاج البروتينات

Isozyme formation

• إذا عني جينات مختلفة بسر يتكامل لين نفس الانزيم (نفس الوظيفة والشا ح) بسر مختلفين بالجينات التي عملتهم

- Products of different genes: (true isozymes)
- The same locus of the gene may have different alleles → allelic isozymes are called **allozymes** (only one form will be present in the individual)
 - e.g. more than 400 distinct forms of **glucose 6-P dehydrogenase** in population
 - Polymorphism: >1% frequency of variation at a single locus



اختلاف في تسلسل الـ (DNA)

ممكنه يكونه عندي أكثر من (form) منه (iso enzyme) ك (individual)
أما ال (alloenzyme) موجود بس واحد ك (individual)

Isozyme formation

- Molecular heterogeneity of enzymes may also be produced after the protein is synthesized (post-translational modification): iso-forms
تعديل بعد تصنيعه بعد انتهاء تصنيعه
- In some cases, all the different forms of an enzyme are present in the same individual
 - e.g. **LDH** has 5 iso-enzymes

Identification of isozymes

الفصل الكهر بائي الهلامي

1. In Agar gel or polyacrylamide gel **electrophoresis**: isozymes have different mobility

LDH, CK and ALP isozymes can be separated by electrophoresis

2. **Heat stability**: one of the isozymes may be easily **denatured** by heat

Bone isozyme of **ALP** (BALP) ^{Ex} بفرق بينهم مثلا صيا الأستري أنا يصيولاه (توضيح)

بكونه (iso Zyme) معين (مفروض قلنا أنه الـ (forms) - بالـ (iso Zyme) بتفرقه يصعب ممكنه الروابط بتفرقه

3. **Inhibitors**: one of the isozymes may be sensitive to one inhibitor

Tartrate labile ACP ^{لا} يعني بكونه في (I) بس بدتتجرب (iso Zyme) معين وعندني 3 مثلا فاله بصير

لـ (تثبيط) بكونه هو الانزيم المرغوب

4. **K_m value or substrate specificity** may be different for isozymes

Glucokinase ¹ has high K_m and **hexokinase** ² has low K_m for glucose

$$iso Zyme = ② + ①$$

Identification of isozymes

5. **Cofactor** requirements may be different for isozymes

- Mitochondrial isocitrate dehydrogenase is NAD^+ dependent
- Cytoplasmic isozyme is NADP^+ dependent

6. **Tissue localization** may be different for isozymes

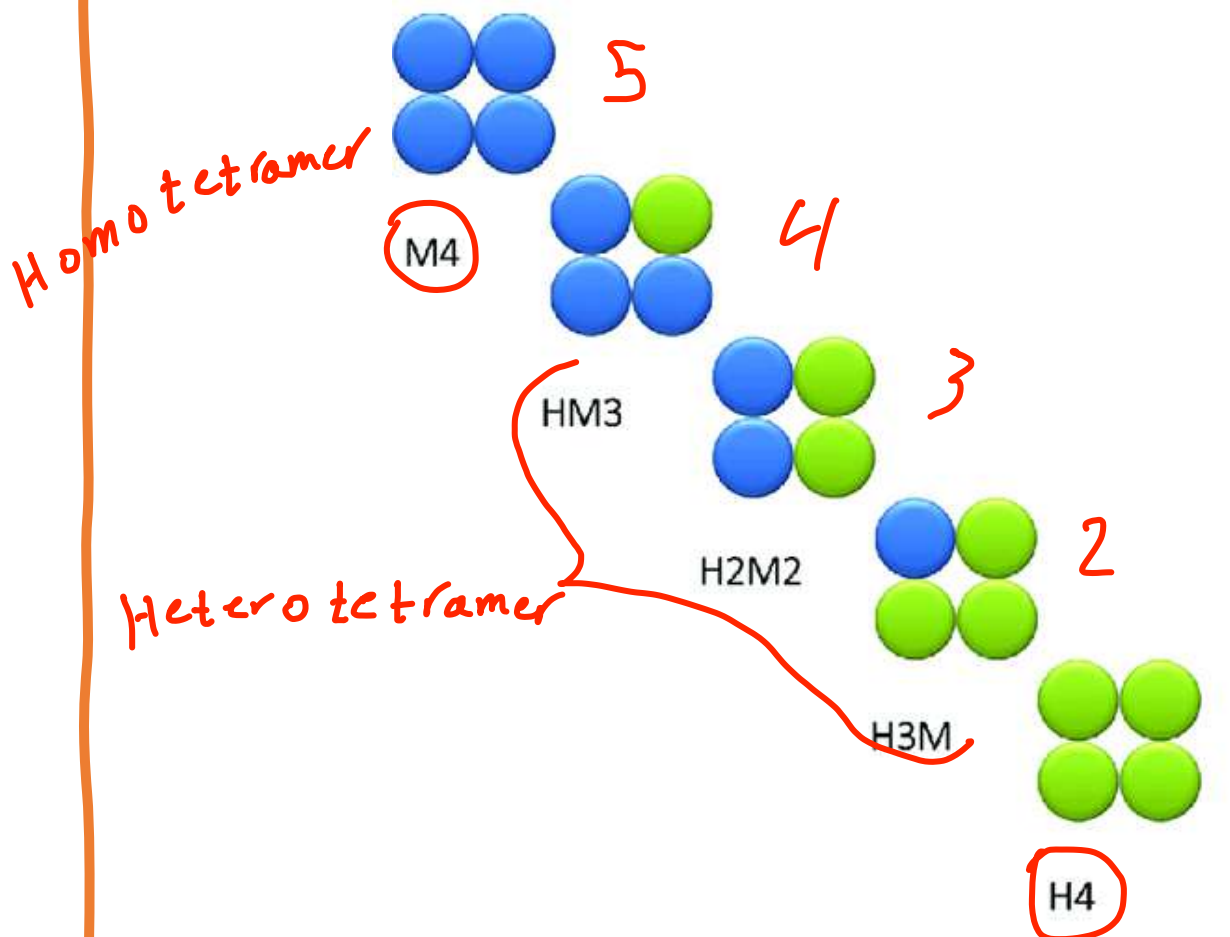
- H4 form of **LDH** is present in **heart**
- M4 variety is seen in skeletal muscle

~~X~~ 7. Specific **antibodies** may identify different types of isozymes

- **CK** iso-enzymes are separated by antibodies

X ش
کتنی بھینا

4 = tetramer *



LDH isomere subunit composition

	M ₄	HM ₃	H ₂ M ₂	H ₃ M	H ₄
Liver	Large dark red circle	Small pink circle	Small pink circle	Very small white circle	Very small white circle
Muscle	Large dark red circle	Small pink circle	Small pink circle	Very small white circle	Very small white circle
White cells	Very small white circle	Very small white circle	Large dark red circle	Very small white circle	Very small white circle
Brain	Very small white circle	Very small white circle	Small pink circle	Large dark red circle	Very small white circle
Red cells	Very small white circle	Very small white circle	Small pink circle	Large dark red circle	Very small white circle
Kidney	Very small white circle	Very small white circle	Small pink circle	Small pink circle	Large dark red circle
Heart	Very small white circle	Very small white circle	Small pink circle	Small pink circle	Large dark red circle

Prevalence of the five isotype in different organs and tissues

Homo tetramer

Applications of isozymes in diagnosis

Clinical Enzymology

- Plasma contains many functional enzymes, which are **actively secreted** into plasma.
- There are a few **nonfunctional enzymes in plasma**, which are coming out from cells of various tissues due to normal wear and tear ^{التآكل} and tear ^{الضعف}.
 - Their normal levels in blood are very low; but are **drastically** ^{بشكل كبير} **increased** ^{انما} during necrosis or disease
↳ the death of body tissue
- Assays of these enzymes are very useful in diagnosis of diseases

(Biological molecules, it locates, in blood, tissue, fluid)

Enzymes as (cardiac) Biomarkers

لما بتعتبرلنا أنه الشخص عنده مرضه معينه، أو لو شخصه أخذ دواء بسببه لي إذا كان الشخص تعالج أو لا فلو مثلا قلب ال (Bm) فالعلاج ناجح

- A **biomarker** is a clinical laboratory test which is useful in detecting dysfunction of an organ

نقص تروية القلب (انخفاض تدفق الدم للقلب)

Different markers are used to:

1. **Detect myocardial ischemia** at the earliest

❖ Commonly used biomarkers for **early detection** of acute myocardial infarction are:



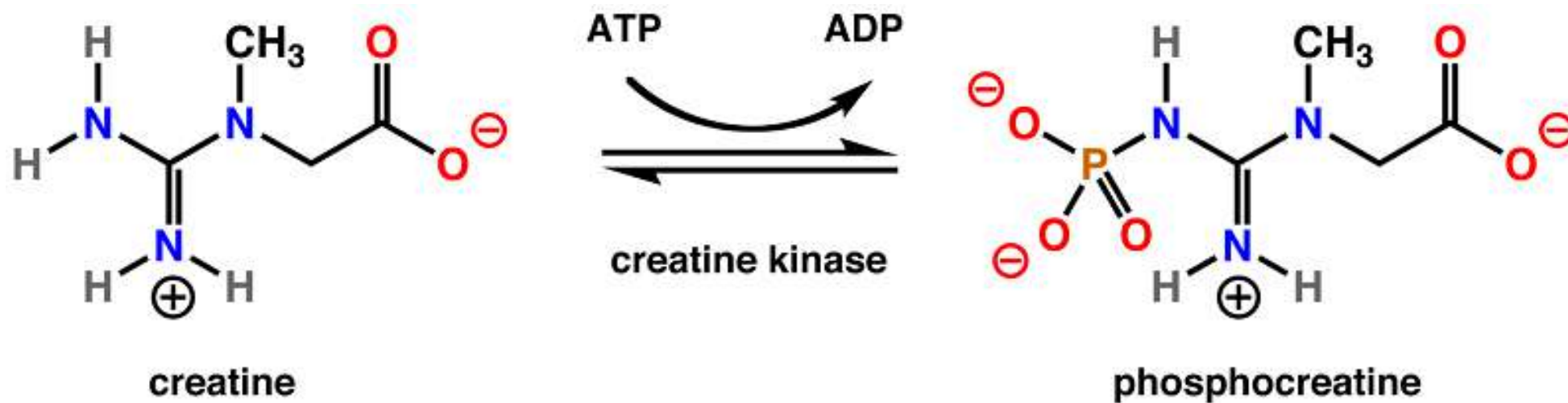
اكتشفنا أنه في امراضه بس تصير عنده تا بنزيد افراز ال (CK) لأنه ال (tissue) مثلا في القلب في مرضه نقص التروية تموت الخلايا اللي ما وصلها دم بالتالي تفرز انزيمات اللي البلازما فنصير الزيادة في بي جي ال (Biomarkers) بيكشف المرضه .

2. **Monitoring** the progression of the condition (Creatine Kinase) عالي فيعرف انه عنده (myocardial infarction)
3. **Predict** the risk in cardiac dysfunction (تنبأ)

CREATINE KINASE (CK)

It catalyzes the creatinine kinase reaction

in the cells and after the cells are dead, it will be secreted to the blood



CK and Heart Attack

- CK value in serum is **increased** in myocardial infarction (الدم)
 - The CK level starts to rise **within 3-6 hours** of infarction
- CK estimation is very useful to **detect early cases**, where ECG changes may be ambiguous (تقدير تضيق القلب ما يكونه دقيقه)
 - A second peak may indicate another ischemic episode

Markers of myocardial infarction

Marker	Onset	Peak ^{القمة}	Duration
CK-MB	3-6 hr	18-24 hr	36-72 hr
Troponins	4-10 hr	18-24 hr	8-14 days
LDH	6-12 hr	24-48 hr	6-8 days
AST	24-36 hr	4-5 d	10-12 days
Myoglobin	1-4 hr	6-7 hr	24 hr

CK and Heart Attack

• The CK level is not increased in hemolysis or in congestive cardiac failure

تكرار (RBC)

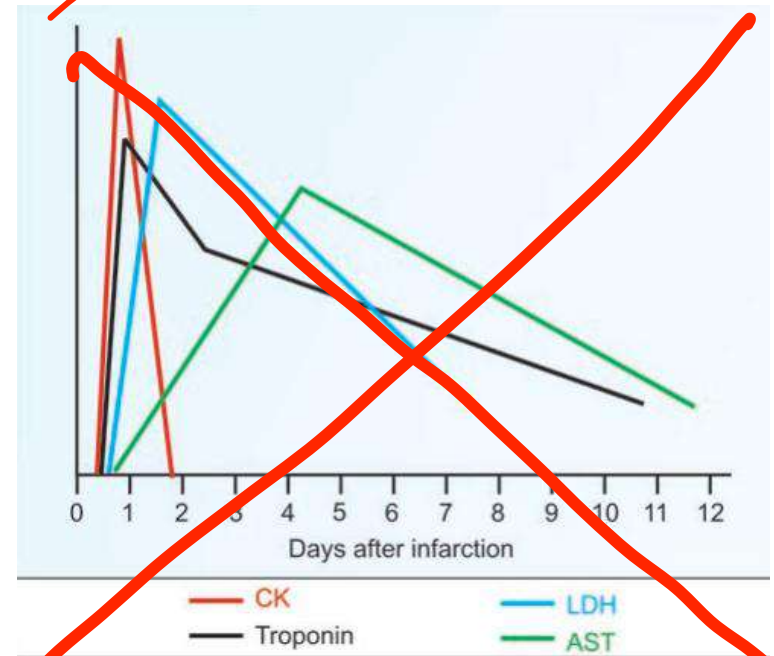
(القلب ما يرفع الدم بكفاءة زي ما هو مطلوب)

- CK has an advantage over LDH → (CK is very specific)
- The area under the peak and slope of initial rise are proportional to the size of infarct

(myocardial infarction)



(بميز بس مشكلة واحدة)



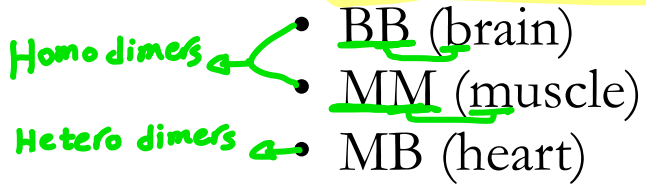
Time course of CK, LDH, Troponin and AST in blood of MI patients

Iso-enzymes of CK

(two subunit) ^{بكون من}

- CK is a **dimer**; each subunit has a molecular weight of 40 kD

- The **3 isozymes of CK:**



ما أجبي بدي أقيده ال (myocardial infarction)
أي وحدة لازم أقيده؟ MB؟ (heart)

- Normally CK2 (heart isozyme) is only 5% of the total activity.
- Even doubling of the value of CK2 (MB) isozyme may not be detected, if total value of CK alone is estimated
- **MB isoenzyme estimation is the best diagnostic marker in MI**

CK isozyme characteristics

Iso-enzyme	Electrophoretic mobility	Tissue of origin	Mean percentage in blood
MM (CK3)	Least	Skeletal muscle	80%
MB (CK2)	Intermediate	Heart	5%
BB (CK1)	Maximum	Brain	1%

Estimation of **total CK** is employed in **muscular dystrophies** and MB isozyme is estimated in MI

ایش بخصه کل الا انواع؟
دانه نسبتہ (MM) کثیر عالیہ

CARDIAC TROPONINS (CTI/CTT)

- They are not enzymes

قالت هاد السلايد اقدموه قراءة
من ركزت على أول نقطتين خصوصا الكلمة الصفراء

- Troponins are now accepted as reliable markers for MI

موثوقة / معتمدة

- ~~•~~ Measurement of cardiac troponins are among the main tests in early detection of an ischemic episode and in monitoring

LACTATE DEHYDROGENASE (LDH)

- **LDH convert pyruvate to lactate** (Normal value of LDH in serum is 100-200 U/L)
- Values in the upper range are generally seen in children
- Strenuous exercise will slightly increase the value
- LDH level is 100 times more inside the **RBC** than in plasma (لأنه RBC ما عنده ميتوكوندريا فبحاجة طاقة)
- Minor amount of **hemolysis** will result in a false positive test

يعني بس اصب عينة الدم واتركها شوي ويصير السكر رح يعمل (release) لـ (LDH)
فبالتالي ممكن بطينه اختبار ايجابي خاطوه هو مش (Specific) زي (CK)

LDH and Heart Attack

- In MI, total LDH activity is increased

- تذکرہ: انہ کا نہ موجود اکثر شریہ فی
(Heart)
- LDH1 (H4) isozyme is increased 5-10 times more

Differential Diagnosis

- Increase in total LDH level is seen in hemolytic anemias, hepatocellular damage, muscular dystrophy, carcinomas, leukemias, and any condition which causes necrosis of body cells
- Total LDH is \uparrow in many conditions \rightarrow LDH isozyme study is important

LDH isozymes

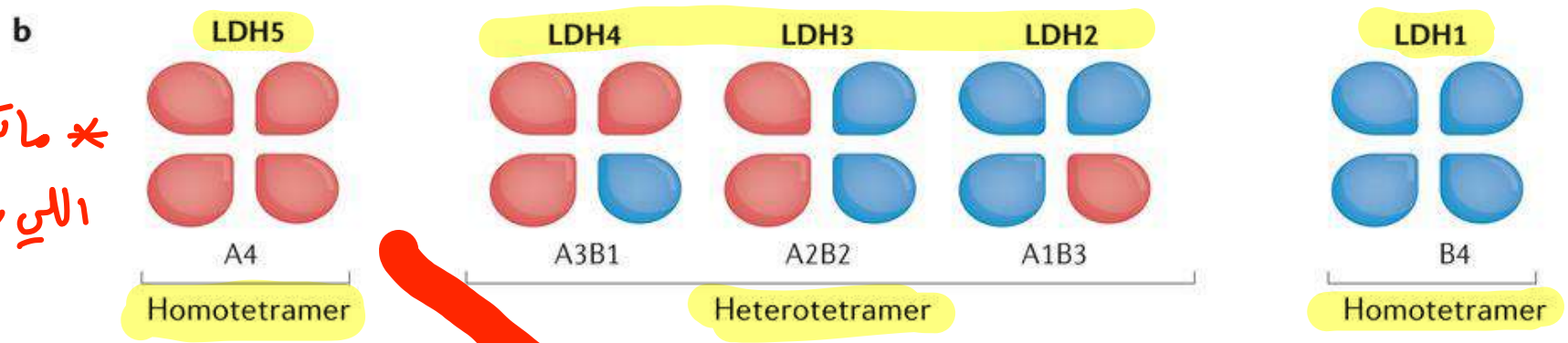
LDH enzyme is a tetramer with 4 subunits (subunit may be either H (heart) or M (muscle) polypeptide chains).

- ❖ H subunit and M subunit have the same molecular weight with minor amino acid variation
- ❖ 5 (Isozymes) combinations of H and M chains are possible
 - LDH1, LDH2, LDH3, LDH4, LDH5
 - All these 5 forms are seen in all persons
 - The isozymes are usually separated by **electrophoresis**
- Normally LDH2 concentration in blood is $>$ LDH1
- LDH has only limited diagnostic value because its non-specific

بمعلومة عامة لا تركز عليها

المعلومة الأهم

* ما تعرفوا غيبه
اللي بالاصفر



c

	LDH5	LDH4	LDH3	LDH2	LDH1
Expressed in	<ul style="list-style-type: none"> Liver Skeletal muscle Kidney (medulla) 	<ul style="list-style-type: none"> Liver Skeletal muscle Kidney (medulla) 	<ul style="list-style-type: none"> Lymphoid tissue Platelets Brain 	<ul style="list-style-type: none"> Heart Red blood cells Kidney (cortex) 	<ul style="list-style-type: none"> Heart Red blood cells Germ cells
Non-malignant serum (% of activity)	8–20%	9–15%	16–25%	27–37%	17–27%
Related diseases	<ul style="list-style-type: none"> Liver disease Skeletal muscle trauma Lung cancer Advanced-stage CRC 	<ul style="list-style-type: none"> Breast cancer Advanced-stage melanoma Advanced-stage CRC 	<ul style="list-style-type: none"> Leukaemia Breast cancer Advanced-stage melanoma 	<ul style="list-style-type: none"> Haemolytic diseases Non-Hodgkin lymphoma Lymphocytic leukaemia Myeloproliferative syndrome 	<ul style="list-style-type: none"> Myocardial infarction Haemolytic anaemia Ovarian cancer Testicular cancer

Isoenzymes of lactate dehydrogenase



H_4 (LDH₁)



H_3M (LDH₂)

Highest levels found in the following:

Heart, kidneys

Red blood cells, heart, kidney, brain

Isoenzymes of lactate dehydrogenase



H_2M_2 (LDH₃)



HM_3 (LDH₄)



M_4 (LDH₅)

Highest levels found in the following:

Brain, lung, white blood cells

Lung, skeletal muscle

Skeletal muscle, liver

Enzyme Profiles in Liver Diseases

Enzymes commonly studied for diagnosis of liver diseases are:

- Alanine amino transferase (ALT)
- Alkaline phosphatase (ALP)
- Nucleotide phosphatase (NTP)
- Gamma glutamyl transferase (GGT)

لفظ
و ترجمه

ENOLASE (Enzyme)

- A glycolytic enzyme

- Neuron-specific enolase (NSE) is an isozyme seen in neural tissues and neuro (Apudomas) → (Neuroendocrine tumors)

- NSE is a **tumor marker** for cancers associated with neuroendocrine origin, ~~small cell lung cancer~~, ~~neuroblastoma~~, ~~pheochromocytoma~~, ~~medullary carcinoma of thyroid~~

Aldolase (ALD)

- A tetrameric enzyme with A and B subunits → 5 isozymes
- A glycolytic enzyme as same as Enolase

✗ { Normal range of serum is 1.5-7 U/L } ما صكتنا (شبهه)

- Drastically ↑ in muscle damages such as progressive muscular dystrophy, poliomyelitis, myasthenia gravis and multiple sclerosis
- It is a very sensitive early index in muscle wasting diseases

Enzymes as Therapeutic Agents

(TA)

حفظ حفظ حفظ

Enzyme	Therapeutic application
1. Asparaginase	Acute lymphoblastic leukemia سوفانولم
2. Streptokinase	To lyse intravascular clot
3. Urokinase	do
4. Streptodornase	DNAse; applied locally
5. Pancreatin (trypsin and lipase)	Pancreatic insufficiency; oral administration
6. Papain	Anti-inflammatory
7. Alpha-1-antitrypsin	AAT deficiency; emphysema

- **Streptokinase** (from Streptococcus) or **Urokinase** (from urine) can lyse **intravascular clots** and are therefore used in myocardial infarction
 بطله في انداد و في ال (Arteries) فيفتح وبعده تروية للقلب
 TA
- **Pepsin** and **trypsin** are given to patients with **defective digestion**
 صعوية هضم
- **Asparaginase** is used as an anticancer drug

Enzymes Used for Diagnosis

Enzyme	Used for testing
Urease	Urea
Uricase	Uric acid
Glucose oxidase	Glucose
Peroxidase	Glucose; Cholesterol
Hexokinase	Glucose
Cholesterol oxidase	Cholesterol
Lipase	Triglycerides
Horse radish peroxidase	ELISA
Alkaline phosphatase	ELISA
Restriction endonuclease	Southern blot; RFLP
Reverse transcriptase	Polymerase chain reaction (RT=PCR)

Handwritten red note: 1. 0.0 P

Handwritten red line: A horizontal line crossing the table rows for Lipase, Horse radish peroxidase, and Alkaline phosphatase.

Handwritten red X: A large red 'X' is drawn over the bottom half of the table, covering the rows for Horse radish peroxidase, Alkaline phosphatase, Restriction endonuclease, and Reverse transcriptase.

Handwritten red note: 1. 0.0 P

Required

- Read on enzyme regulation (allosteric enzymes) the difference between homotropic and heterotropic effectors.

اقراء و فهم منه ليفند كوت .