23

Faculty of medicine – JU2018

◉ Sheet ○ Slides

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Recapitulation:

Enzyme efficiency affected by:

1) Kcat: (turnover number) the ability of an enzyme to convert a substrate to a product upon binding to it.
2) Km: the substrate concentration at which V_0 is half of V_{max}.

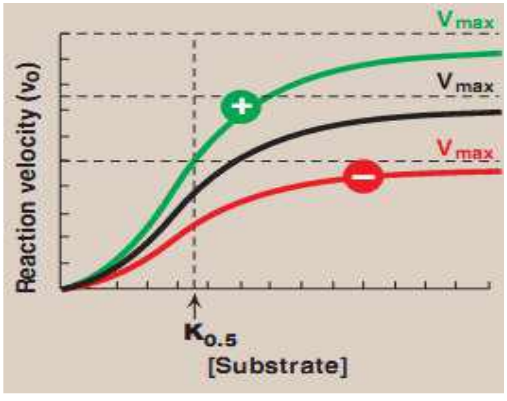
   We can measure an enzyme efficiency using the following equation:

- Enzyme efficiency= Kcat/Km

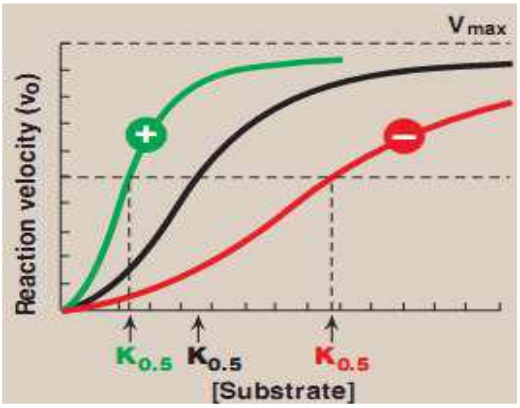
There are two possibilities that may occur if an allosteric modifier binds to an allosteric enzyme:

1) **V system:** upon binding to allosteric modifier, the V_{max} changes however, the $K_{0.5}$ remained constant.
2) **K system:** upon binding to allosteric modifier, the V_{max} remained constant however, the $K_{0.5}$ change.

V- system



K- system



Non-specific inhibitor

Non-specific inhibitor does not target any enzyme in specific, it effects enzymes in general. *

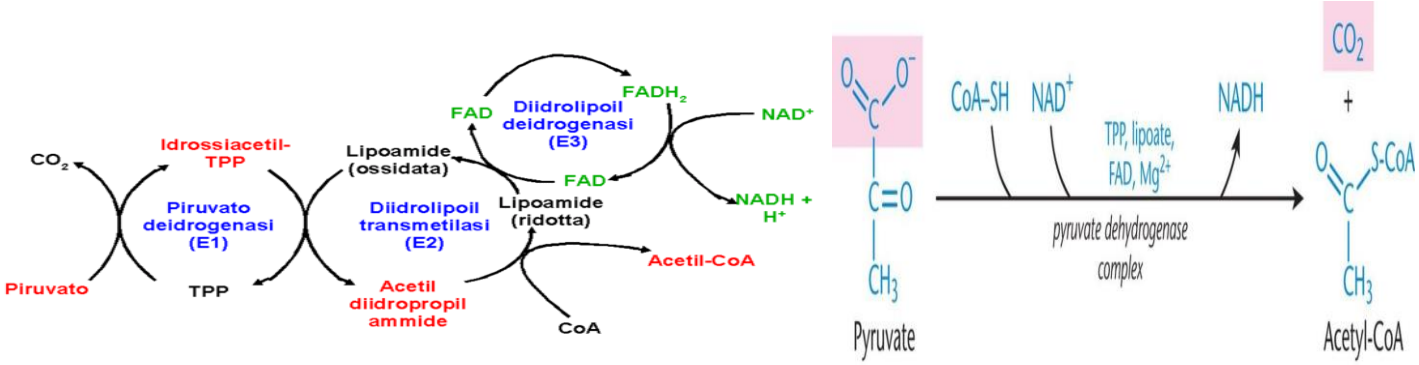
- Enzyme concentration is regulated in three ways:
    1. Synthesis of isozyme: within the same cell two isozymes catalyze the same reaction but they have different catalytic efficiency and regulatory mechanisms.
    2. Enzyme synthesis at gene level (gene expression): When a certain gene is expressed at a much higher frequency, the number of enzymes within the cell will increase (this is because most enzymes are proteins) and in turn the V_{max} will increase.
    3. Enzymes degradation by proteases.
- They are comparatively slow mechanism for regulation enzyme concentration (hour-weeks), but cells go through a process known as fast regulation (phosphorylation, dephosphorylation and cleavage.

Compartmentalization

- A reaction between substrate and enzyme occur by collision and this collision must occur at the proper orientation (the precise angle).
- Cells tend to enclose certain enzymes and substrate in small compartments such as the lysosome. This is considered advantageous for many reasons, it will decrease the area of the reaction so we limit diffusion and increase the frequency of collision between the enzyme, increasing the probability for enzyme to find substrate so enzyme can easily find the substrate, bind to it and convert it to the product and create an optimum microenvironment for the enzymes of that compartment. Lysosomal enzymes need an acidic environment in order to function.
- When enzymes are placed in a small compartment it will not change Km or V_{max} but it will increase the rate of reaction.

Enzyme complexing

- Most metabolic pathways in cells occur in a series of reactions
- For Example when substrate “A” binds to enzyme “A” the substrate is converted to product “B”, then product “B”, now substrate “B”, diffuses until it randomly collides with enzyme “B” and is converted to product “C” then substrate “C” diffuses to the third enzyme and so one.
- So, the formation of product is limited by diffusion and the frequency of collision between the enzyme and the substrate. This will increase the time taken to form a product.
- As a result, most cells combine more than one enzyme in a single large unit with multiple distinct active sites. The enzyme quickly reacts by remaining within proximity of this large unit. It does not leave this large unit instead it just moves from one enzyme to the next. So, the time taken for a reaction to proceed is decrease substantially. Diffusion is also reduced. The reaction, however, is limited by the first collision.
- For example, pyruvate dehydrogenase in is composed of three enzymes. Decarboxylation, oxidation and transfer of the acyl group to CoA. Each enzyme catalyzes certain reaction and has its own product. The result, however, is converting pyruvate to acetyl-CoA)

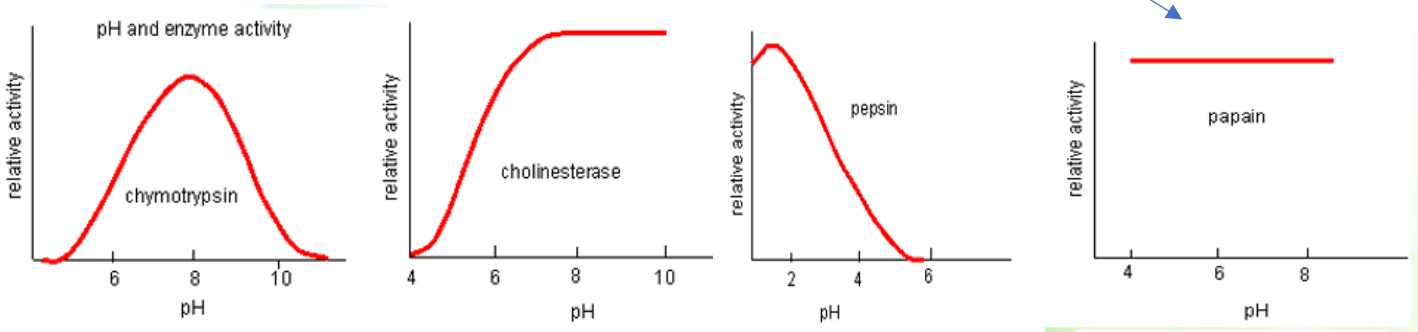


Another effector in protein's function:

1. **Temperature:** The rate of reaction increases with the increase of temperature. This due to the increase of the kinetic energy of molecules. As the kinetic energy increases, the frequency of collision between enzyme and substrate increases.
   However, excess temperature disrupts the bonds that stabilize the structure of the enzyme, so it is denatured.
   Each enzyme has an optimal temperature in which the most efficient configuration takes form.
   The optimal temperature varies between different organisms, for example the optimal temperature for obtaining the most efficient configuration for proteins in human body is 37 however, the optimal temperature of TAQ protein, which is found in thermophilic bacteria, is 65.

2. **pH:** alters the protonation of the substrate and/or enzyme and its binding. The effect of pH is enzyme dependent.

Optimal pH of some enzymes



Chymotrypsin: optimal pH is 8, which is like the intestinal pH, because we have bile acid which neutralizes the stomach acid.

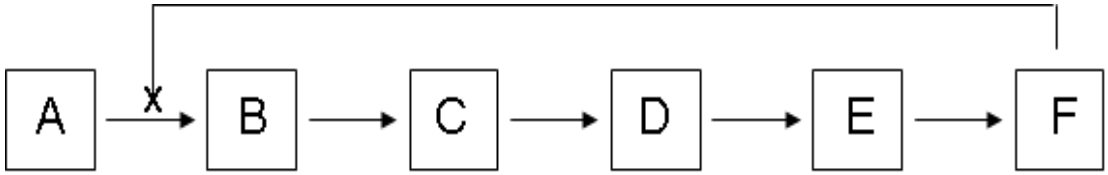
Cholinesterase: optimal PH is 7.5.

Pepsin: optimal PH is 2 because it exists in the stomach, which contains hydrochloric acid.

Papain: Exist in papaya fruit but it isn't affected by the PH.

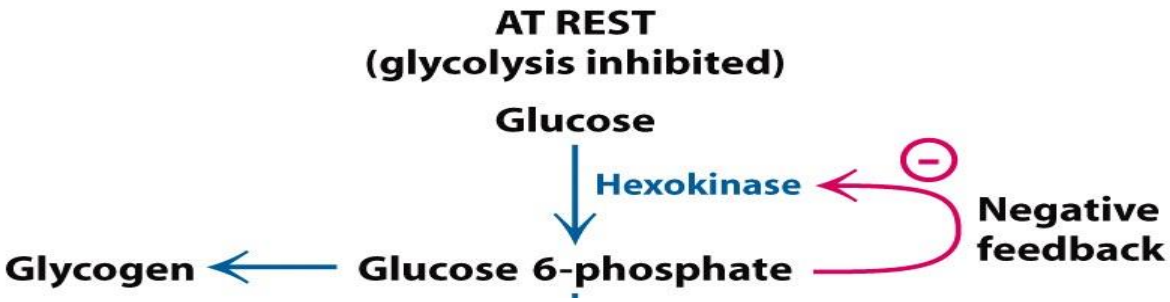
Modes of regulation:

1. **Feedback inhibition (negative feedback):** an enzyme present early in a biochemical pathway is inhibited by a late product in same pathway.

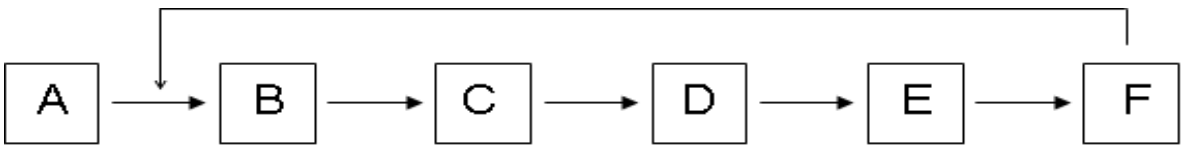


This pathway must be regulated if there is an abundance of A, we need to speed up the reaction. However, if F is in high presence, then we reaction must stop. F will bind to the enzyme that will catalyze the very first reaction.

- If the cell has a lot of ATP, so why does it spend time producing of ATP?
- ATP inhibits hexokinase when it binds to it regulatory site, so the reactions is inhibited.
- Sometime the product inhibits the enzyme that releases it, this known as **product inhibitor**.
  For example: Glucose-6-phosphate can also inhibit hexokinase.

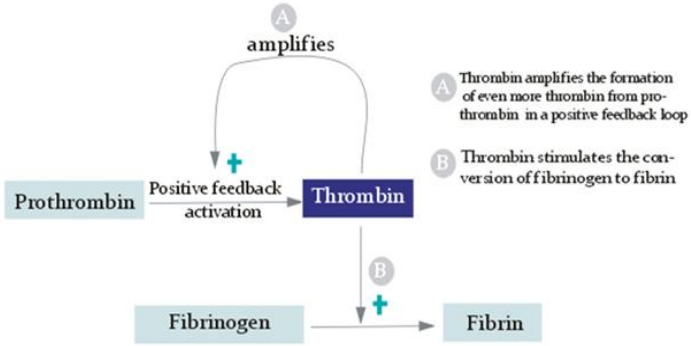


2. **feedback activation (positive feedback):** The Product in the reaction reinforces the activity of an enzyme that precedes the product in the pathway.



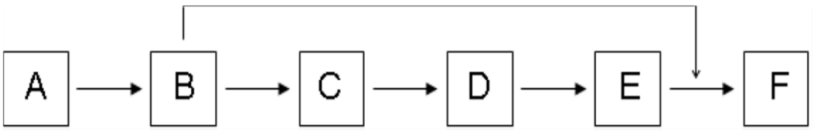
Example: blood clotting in the case of an emergency, this mean our body needs to amplify the reaction through: (the formation of fibrin)

- Formation of thrombin speeds up (positive feedback) the conversion of prothrombin to thrombin (product activator).
- Thrombin stimulates the conversion of fibrinogen to fibrin (feed-forward activation)



Positive feedback activation of more prothrombin into thrombin

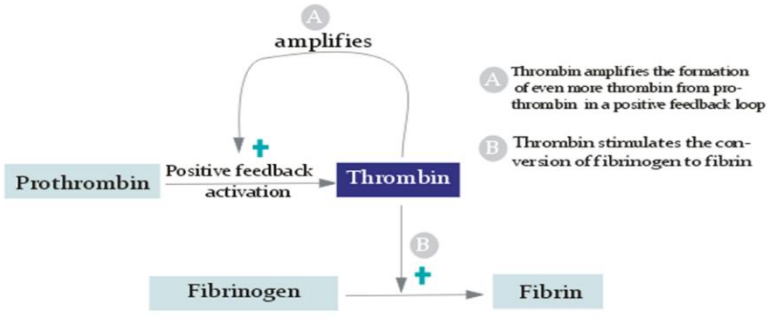
3. **Feed-forward regulation**: a substrate produced early in a pathway activates an enzyme downstream of the same pathway. For example, Thrombin partakes in feed forward activation by activating the reaction which converts fibrinogen to Fibrin.



If we increase the formation of F the concentration of E decrease and the equilibrium moves to right, so more A convert to B.

Thrombin partake in feed-forward activation by enhancing the reaction in which fibrinogen gets converted to fibrin.

- Blood clotting



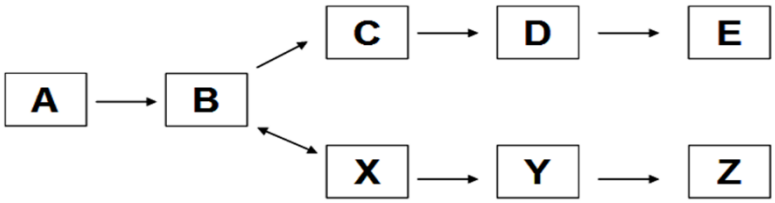
Positive feedback activation of more prothrombin into thrombin

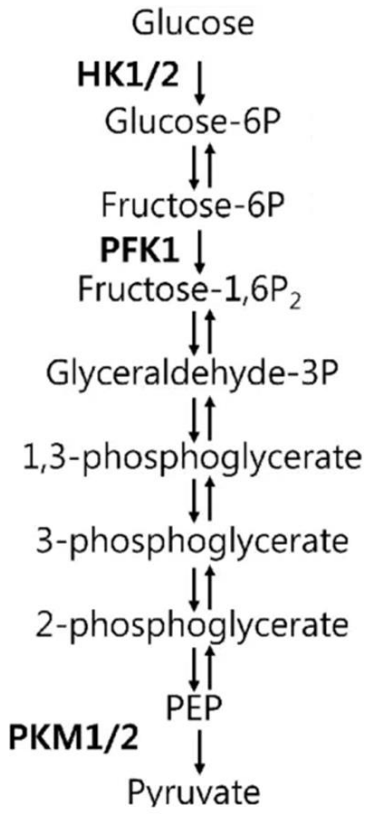
- Poisoning: A produces high amounts of every other substrate so that the poison can be removed from the body as quickly as possible

A 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.

- Committed steps are exergonic reaction.
- For example, the committed step for making product E is (B → C), not (A → B)

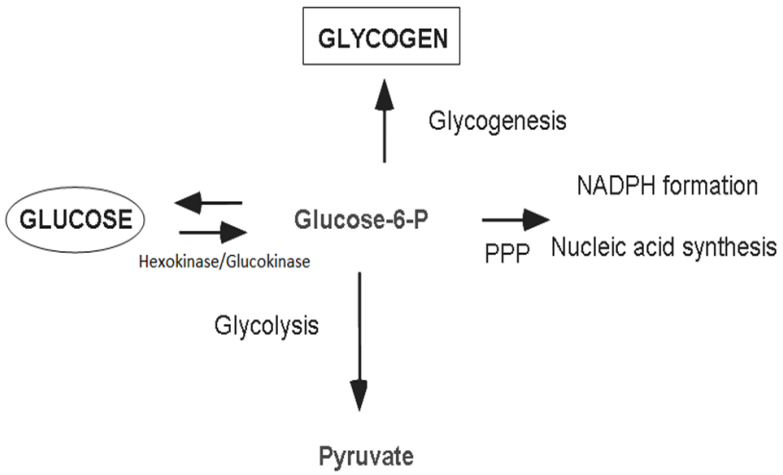




Glycolysis

The commited st ep in this case not HK1/2 this is because many other pathways can occur during this step. The commited step in the case of glycolysis is PFK1 because after the occurance of this step , Fructose-1,6P_2 is commited to the continuation of Glycolysis.

Note that: Glyceraldehyde-3p is can convert to Dihydroxyacetone, but it is not the main pathway.



Rate-limiting Reaction:-

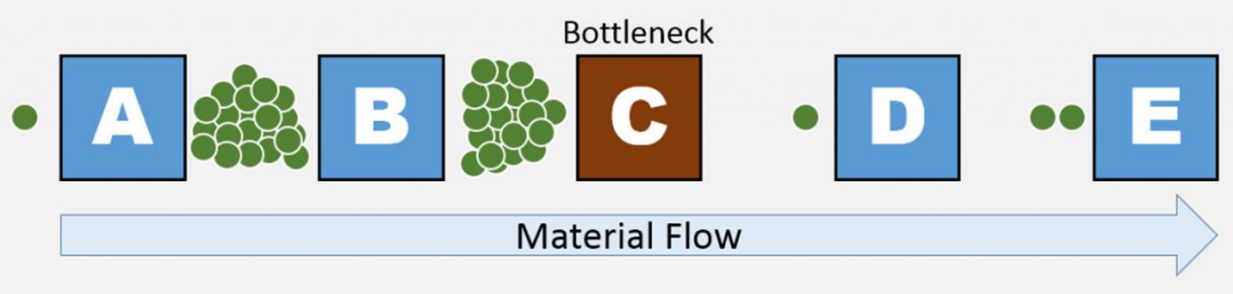
Rate limiting reaction slow down rate of reaction because: They require high energy, strict regulation of enzymes, high km value of enzyme towards its substrate.

These reactions are also usually, but not necessarily, commited steps.

The rate-limiting step is sometimes called the bottle neck step because the reaction proceeds in its entirety undisturbed, however at the bottle neck the is dependent on the continuation of that step.

Rate-limiting reactions occur due to the dependancy on high amounts of energy in order for the reaction to proceed. This means that the reaction will only proceed if the cell is in absolute need of this enzyme.

- During glycolysis for example, the first reaction that takes place (HK1/2) is a rate-limiting reaction due to its dependence on ATP.



Enzymes in disease and diagnosis

The presence of enzymes in serum indicates that tissue or cellular damage.

The measurement enzyme amount in serum is of diagnostic significance.

Examples:

The amino transferases: alanine transaminase, ALT and aspartate aminotransferase, AST

Lactate dehydrogenase, LDH

Creatine kinase, CK (also called creatine phosphokinase, CPK)

Enzymes are of extreme importance when diagnosing patients.As established in earlier lectures enzymes in different tissues are unique. When a tissue gets damged, the cells in that tissue lyse in a process known as necrosis. When necrosis takes place all of the contents of that cell are released into the blood. Through blood sampling doctors can tell which tissue in specific is damaged because cells carry enzymes that are unique to .that cell

ALT/AST

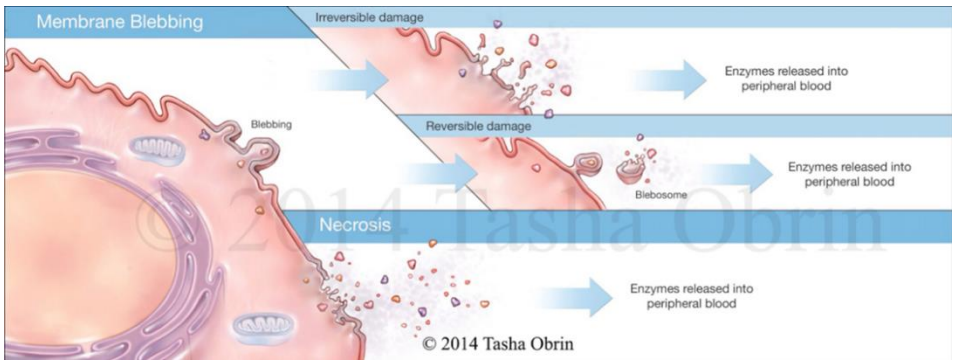
The typical liver enzymes measured are AST and ALT.

ALT is predominantly in hepatocytes.

The ratio of ALT/AST is diagnostic.

Liver disease/damage (not of viral origin) < 1.

Viral hepatitis > 1.

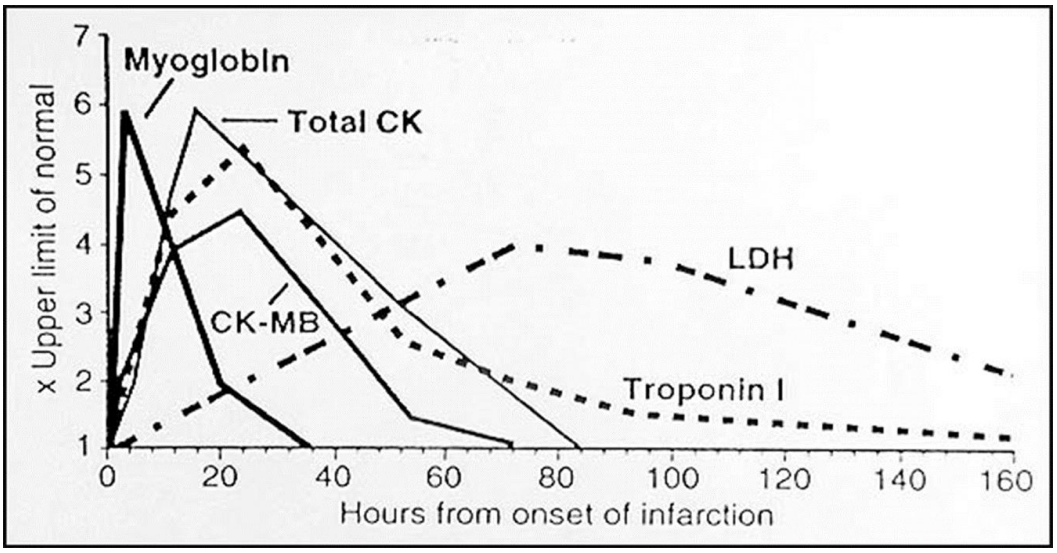


LDH

A comparison of serum levels of 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



When Heart muscles get damaged, they release their content into the blood through a process known as necrosis. We can use heart muscle enzymes to diagnose heart attack from its concentration in blood, the first protein comes up is Myoglobin protein, which is a muscle enzyme (heart is composed muscle tissue and muscles have plenty of myoglobin) but myoglobin isn't a unique to the heart we cannot use it as a marker to detect myocardial infarction (heart attack) because myoglobin is found in all muscle tissue. In the case that the ratio of myoglobin is high, it doesn't necessarily mean that heart cells are going through necrosis. We could, however, use LDH. Although LDH is found in other muscle cells the LDH found in the heart is unique to the heart. So, we could use the LDH serum ratio to detect and diagnose myocardial infarction.