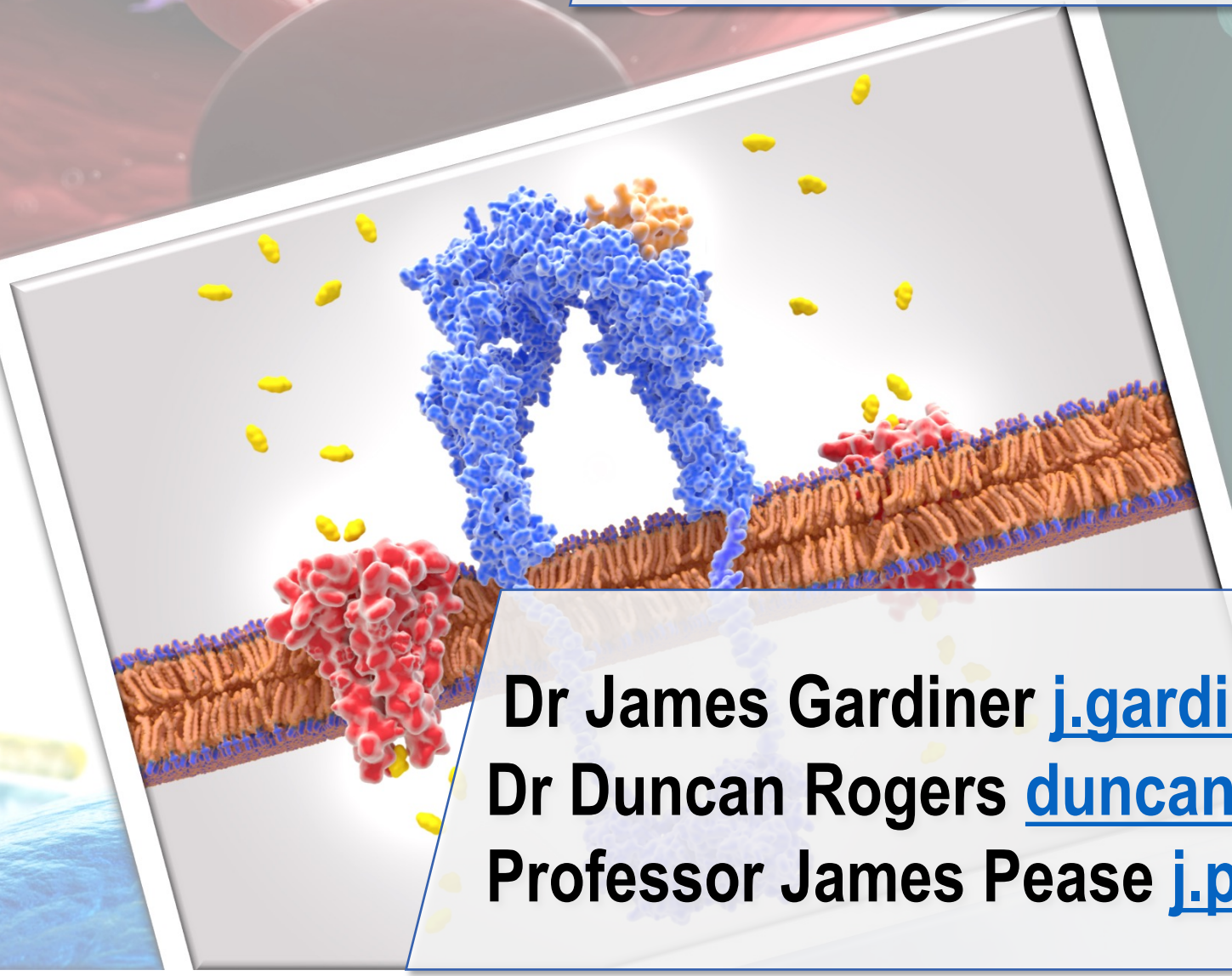
IMPERIAL

Creatine Kinase - tutorial



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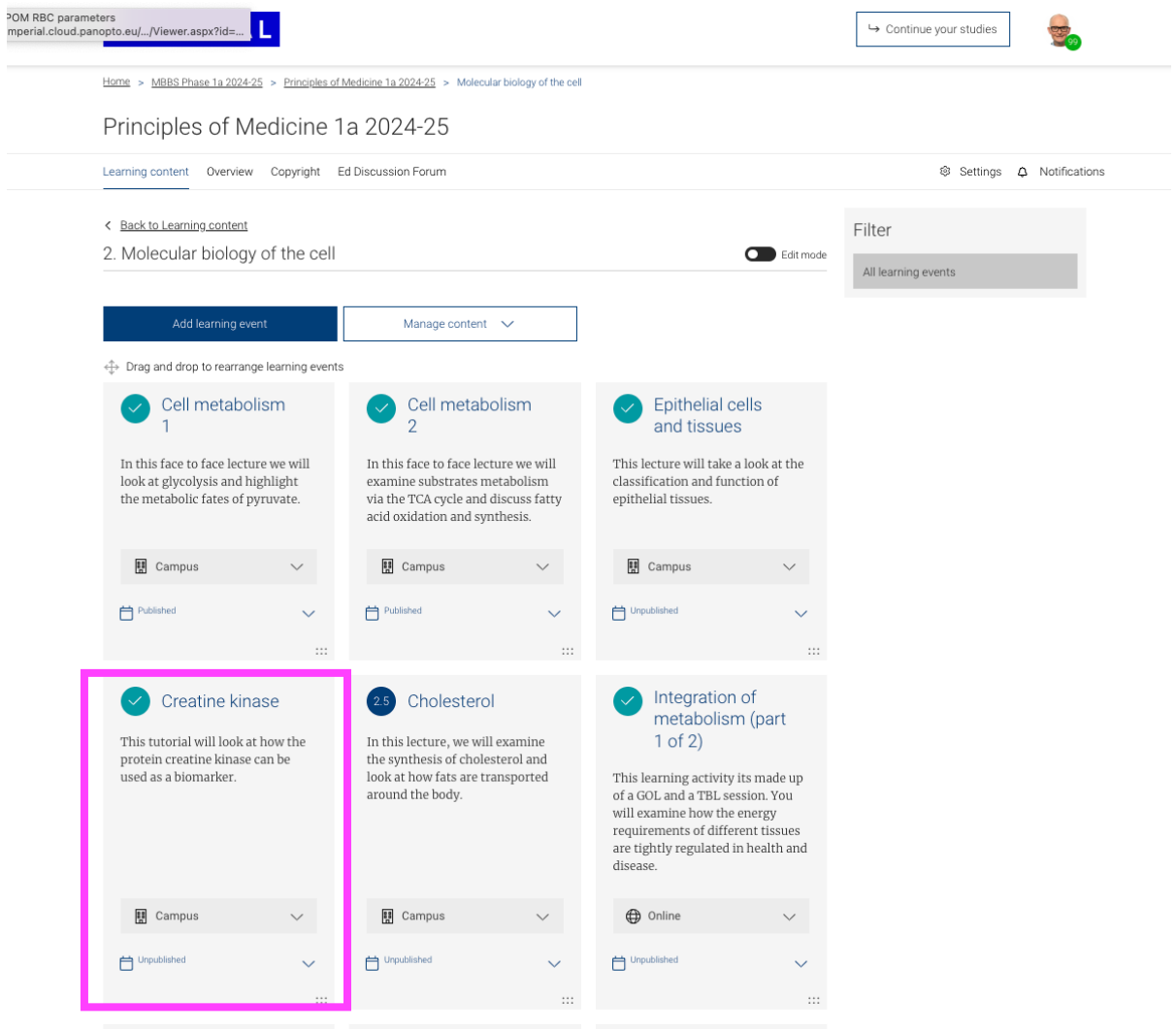
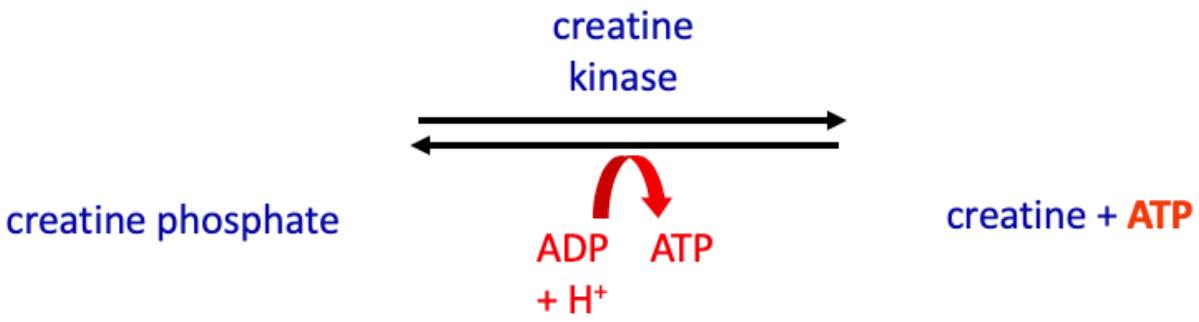
Insendi: MBC – Creatine kinase



Mr Adamson

- Mr. Ronald Adamson, 62, was a very keen gardener. Gardening had become rather an obsession – his lawn had to be mowed three times a week in summer.
- On a particularly hot summer's day, his electric lawn mower jammed. He tried to heave it apart, sweating profusely – not even stopping for a cigarette. His wife called out to offer him a cup of tea in the shade.
- When he hadn't touched his drink some 10 minutes later, she went out to look for him.
- She found him collapsed over the mower, initially panicked, but then called an ambulance.

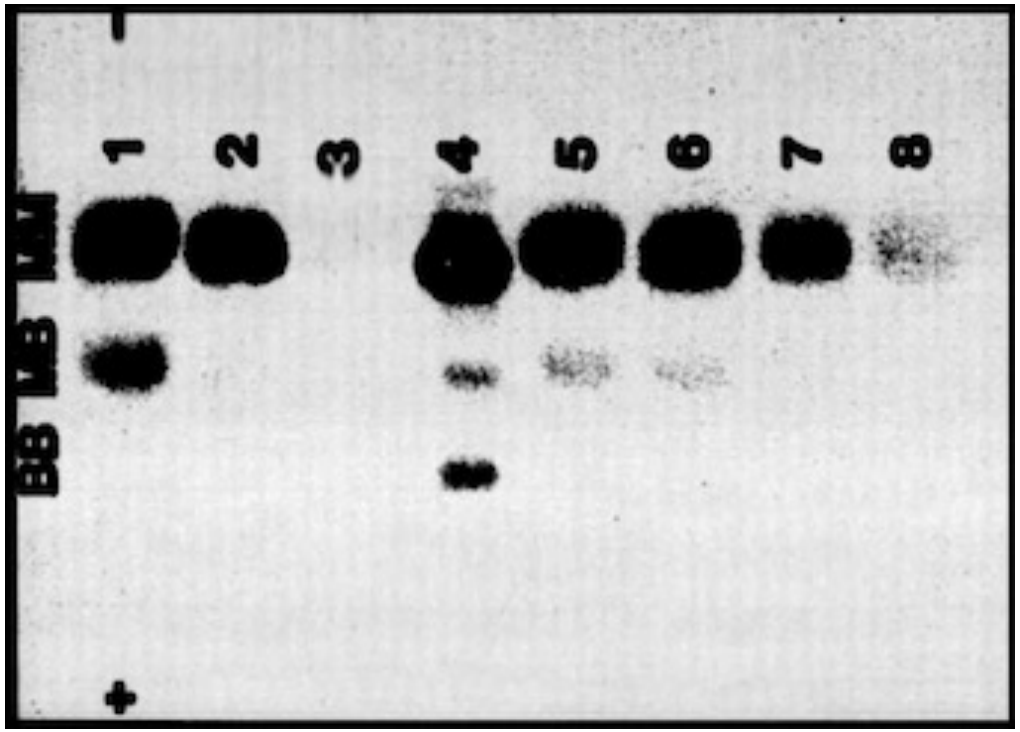
Creatine kinase - 1



$\Delta G^{\circ\prime}$ (hydrolysis) = -31 kJ/mole (ATP) & -43.1 kJ/mole (CP)

- Upon arriving in hospital one of the diagnostic blood tests was analysis for **creatine kinase**.
- You might recall the function of creatine kinase from the earlier LOL event **Cell Metabolism 1** , which is recapped (left). 1-POM-1-1
- Creatine kinase (CK) is probably present in all cells but is present in particularly high concentrations in **muscle cells** and **brain cells**.
- Following the damage or death of such cells CK is released into the **circulation**

Creatine kinase - 2



Foreback and Chu, CRC Critical Reviews in Laboratory Science, 1981.

MM can be seen to move the furthest towards the negative electrode.

- Three dimeric isoenzymes of creatine kinase are known in humans.
- The two different subunits **M** and **B** provide the following isoenzymes: **MM**, **MB** and **BB.**
- The only human tissue where the **MB form** is found is the myocardium, where **MB** represents about 15% of total creatine kinase, the rest being **MM**.
- The three isoenzymes can be separated by electrophoresis on cellulose acetate strips as shown above (see the **Haem: Haemoglobin p**ractical class where we perform this technique). 1-POM-1-5

Questions to be addressed (in groups)

- In what tissues is CK present at high levels?
- When and why is CK found in the blood?
- What causes the plasma membrane of myocardial cells to become leaky?
- How might you determine CK activity?
- Why might the three isoenzymes be separated by electrophoresis?
- How might one establish a diagnosis of myocardial damage?
- Does an increase in serum CK activity relate to the size of myocardial damage?
- What is the time course of serum CK after a myocardial infarction?
- What other markers can be used for diagnosis of myocardial damage?

Take home messages - 1

- In what tissues is CK present at high levels?
- When and why is CK found in the blood?
- What causes the plasma membrane of myocardial cells to become leaky?

What is a myocardial infarct?	The death of heart muscle cells.
Why do the cells die?	Lack of oxygen.
Why is there a lack of oxygen?	**Blockage** of the cardiac arteries**.** This process is termed **atherosclerosis** and you might like to ponder on what kind of things might be risk factors for its early development.

Take home messages - 2

- In what tissues is CK present at high levels?
- When and why is CK found in the blood?
- What causes the plasma membrane of myocardial cells to become leaky?

Why do cells need oxygen?

How do they use it?

Why do cells die without it?

- A semi-permeable membrane separates the inside from the outside of a cell, actively excluding some things e.g. **Na^+ ions**.
- This needs a protein pump in the membrane (**ATPases)**
- They use energy in the form of adenosine triphosphate (ATP) to pump ions. 1-POM-1-3
- How is ATP generated? Via **glycolysis**, the **Krebs Cycle** and eventually **oxidative phosphorylation**. 1-POM-1-1 and 1-2
- The end point of the process requires atmospheric oxygen, hence if there is less oxygen supplied to a cell there is less ATP, pumps do not function, ion balance is lost and cells **die**.

Take home messages - 3

- In what tissues is CK present at high levels?
- When and why is CK found in the blood?
- What causes the plasma membrane of myocardial cells to become leaky?

Cell contents are released when they are **dying**, i.e. proteins that should be held inside against concentration gradients appear in the serum.

Therefore the levels of many proteins including creatine kinase (many others as well such as lactate dehydrogenase) in serum can be used as indirect indicators of **cell death**.

CK is present in all cells at very low levels but is at high concentrations in metabolically very active tissues including the brain, heart and skeletal muscle.

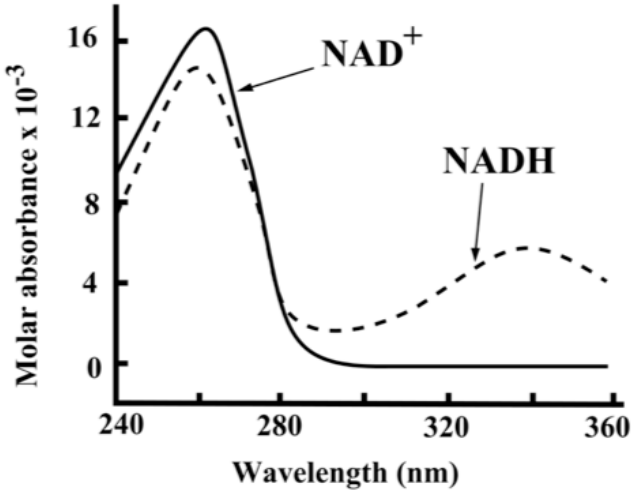
Take home messages - 4

- How might you determine CK activity?

creatine phosphate + ADP $\xrightarrow{CK}$ creatine + ATP

D-glucose + ATP $\xrightarrow{\text{hexokinase}}$ ADP + G6P

G6P + $NADP^+$ $\xrightarrow{\text{G6P dehydrogenase}}$ 6-PG + NADPH + H^+

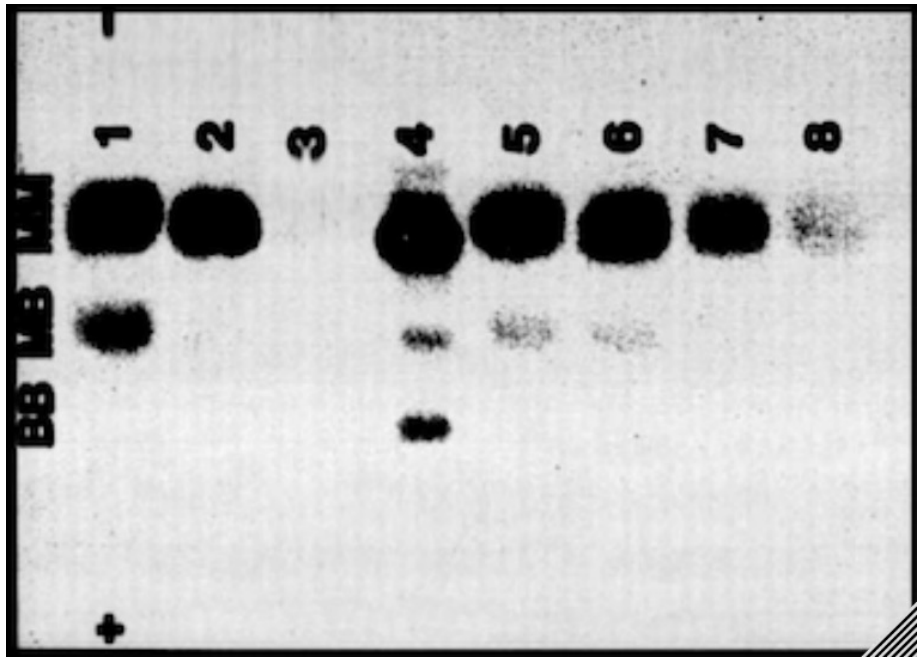


Absorption spectra of NAD^+ and NADH.

- CK activity in the serum can be detected by a coupled assay (left) leading to the generation of detectable products.
- Recall from **PoM primer - Introduction to Laboratory Techniques** that NADH (and NADPH) have absorption spectra distinct from NAD^+ (and $NADP^+$).

Take home messages - 5

- Why might the three isoenzymes be separated by electrophoresis?



- CK is a protein made from two subunits or monomers i.e. it is a **dimer.**
- The two monomers are coded for by two different genes.
- These generate two different monomer **isoforms** "B" and "M".
- The two monomers have approximately the same molecular but differ in their pI (isoelectric point).
- This means that they can be separated by charge)

1-POM-5-3

Take home messages - 6

- How might one establish a diagnosis of myocardial damage?

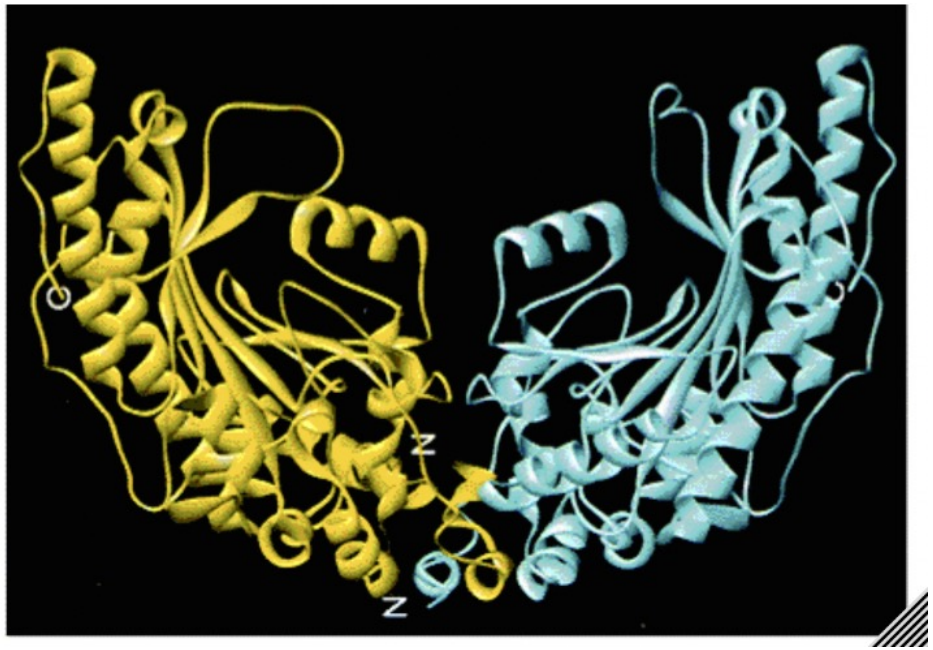


Figure 2. A n MB dimer of creatine kinase. The two monomers are coloured yellow and blue.

- Monomers associate with one another to produce active dimers
- If both genes are expressed in a cell, three final dimers are possible, "**BB**", "**MM**" and "**MB**".
- The Brain **only** expresses the B gene and hence makes only B monomers and so only the BB form can be generated.
- Conversely the MM form is the **only** one made in skeletal muscle cells (useful in diagnosis of the extent of skeletal muscle damage in muscular dystrophies).
- The only tissue where **both** genes are expressed is cardiac muscle cells. They therefore make all three dimers including the hybrid BM form.
- Thus, death of **cardiac muscle fibres** can be determined if the BM isoform of CK can be detected in the serum.

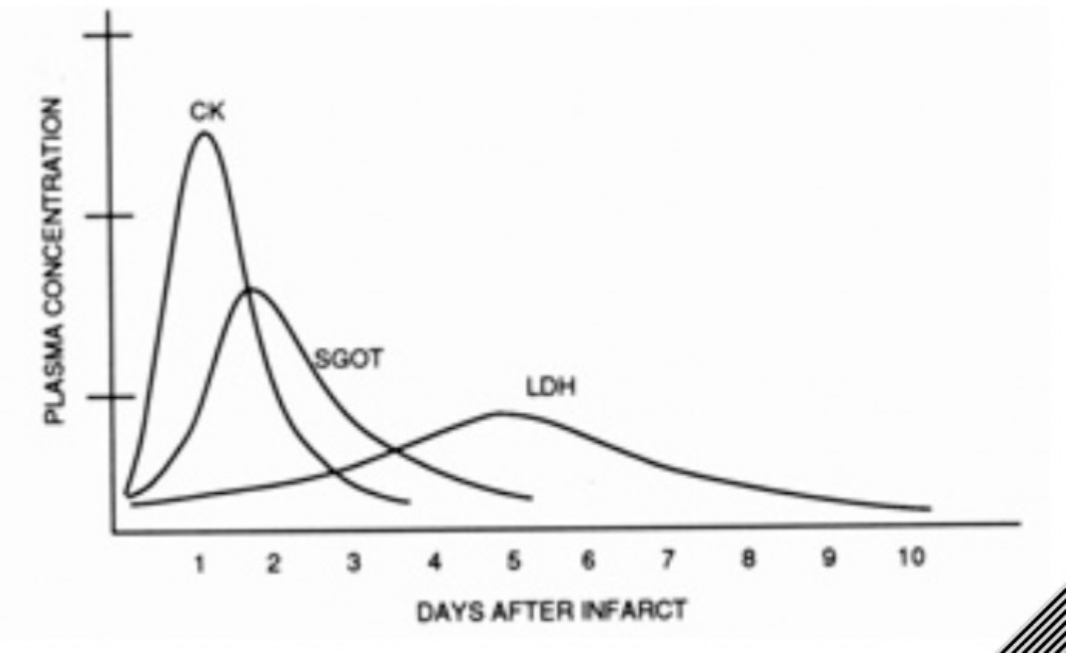
Take home messages - 7

- Does an increase in serum CK activity relate to the size of myocardial damage?

- The levels of CK BM isoform in the serum are directly proportional to the amount of cell death in the heart.
- This is because each myocyte can be considered to be approximately of equal volume (they have equal likelihood of dying independently of their size)
- So, as each cell dies it releases a “quantum” of CK into the extracellular fluid and thence into the serum.

Take home messages - 8

- What is the time course of serum CK after a myocardial infarction?
- What other markers can be used for diagnosis of myocardial damage?



SGOT - serum glutamate oxaloacetate transaminase

LDH - lactate dehydrogenase 1-POM-1-1

Cardiac troponin - Cardiac troponin I and troponin T only present in the heart.

Appearance in the serum is a specific marker for cardiac infarction (48h after infarction and persisting for 5 days).