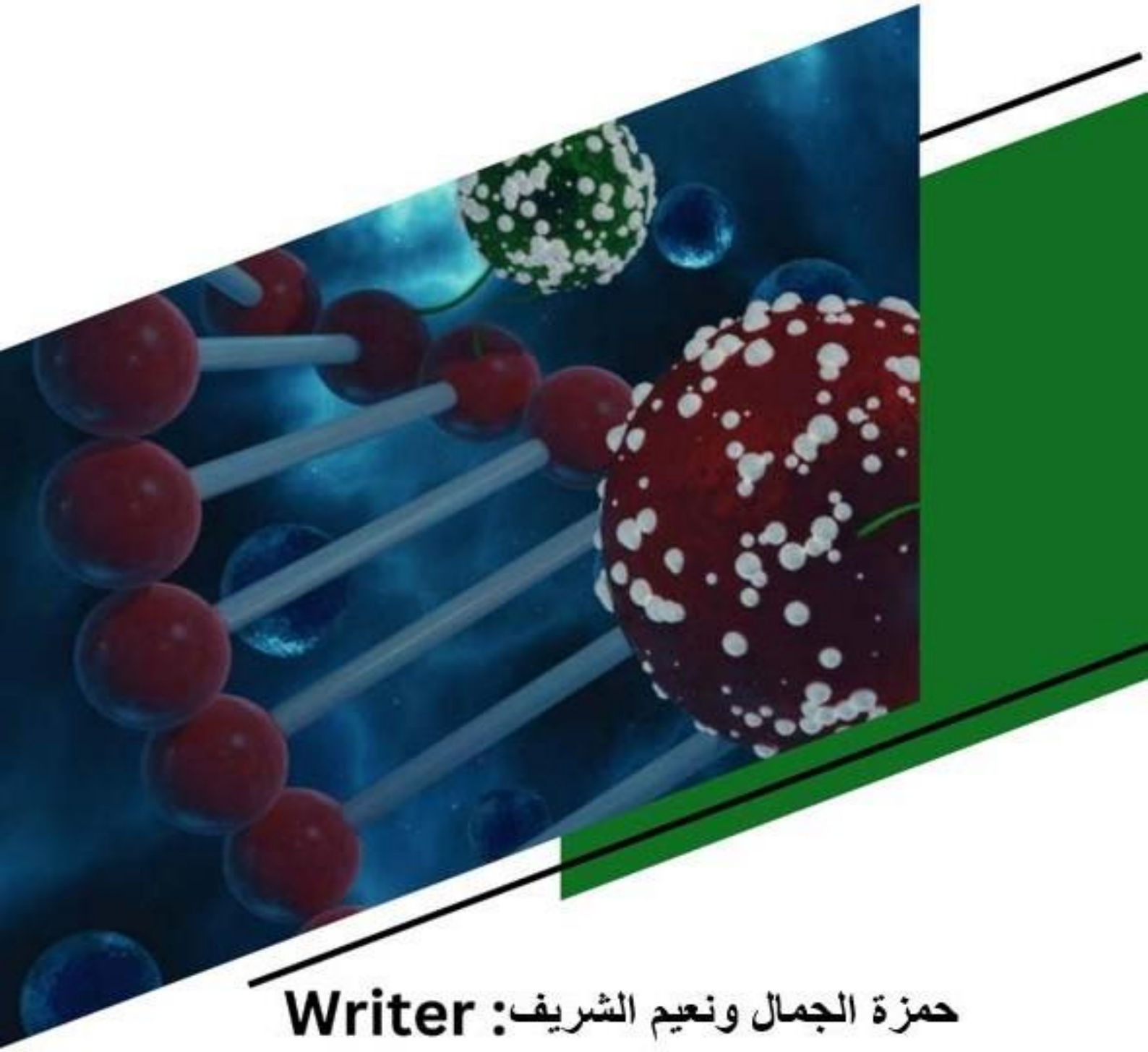
Doctor 021

METABOLISM

Sheet no. 12



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REGULATION OF GYCOLYSIS:

Regulation happens on the 3 irreversible steps which are:

1- Conversion of glucose to glucose 6 phosphate (glucokinase and hexokinase)
2- Phosphorylation of fructose 6 phosphate to fructose 1-6 bisphosphate (phosphofructokinase)
3- Conversion of phosphoenolpyruvate to pyruvate (pyruvate kinase)

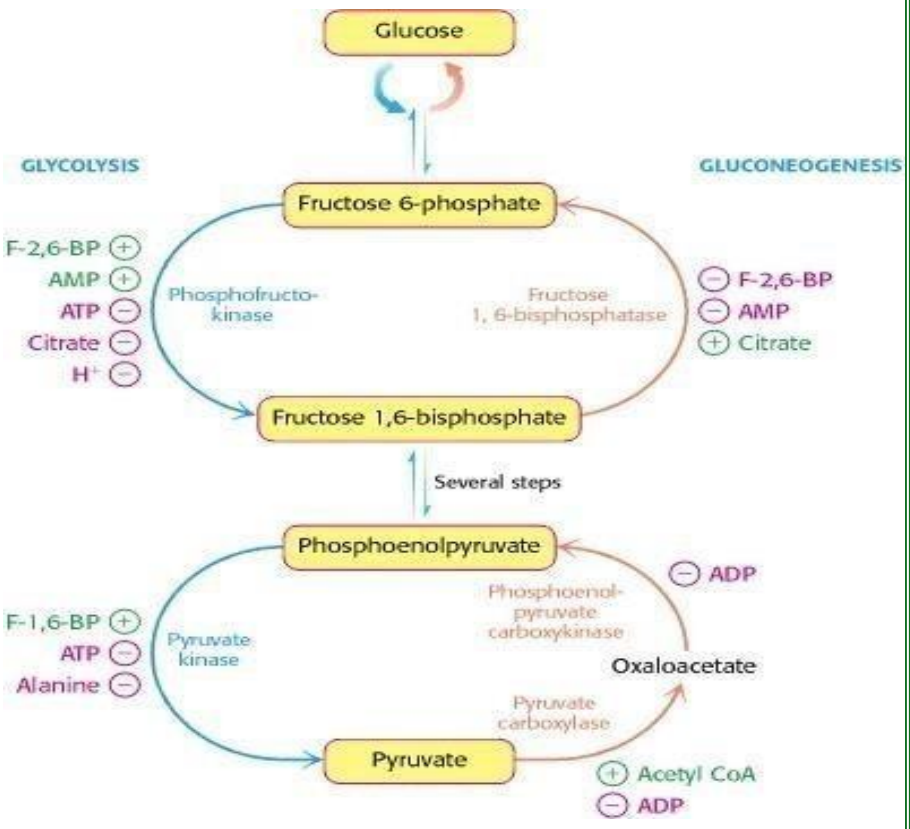
REGULATORS OF PFK AND PK:

We have different ways of regulation.

In the left side of the picture, we have the regulation of Glycolysis while the right side explains the regulation of Gluconeogenesis.

As we see in the picture inhibitors of Glycolysis are activators of Gluconeogenesis and vice versa.

And now darling lets rock this picture….



1- Phosphofructokinase inhibitors and activators:
   A- Fructose 2,6 bisphosphate is an activator if it is a product then it will be an inhibitor.
   B- AMP: it indicates low energy state in the cell so when energy level is low in the cell the cell asks PFK to breakdown glucose in order to generate energy so PFK activates glycolysis.
   C- ATP is an inhibitor because it indicates high energy state and it activates the storage of glucose as glycogen or other shapes.
   D- Citrate (from Krebs cycle) : high amounts of citrate means that TCA cycle is working a lot and there is high production of energy so there is no need to activate more breakdown of glucose. (inhibitor)

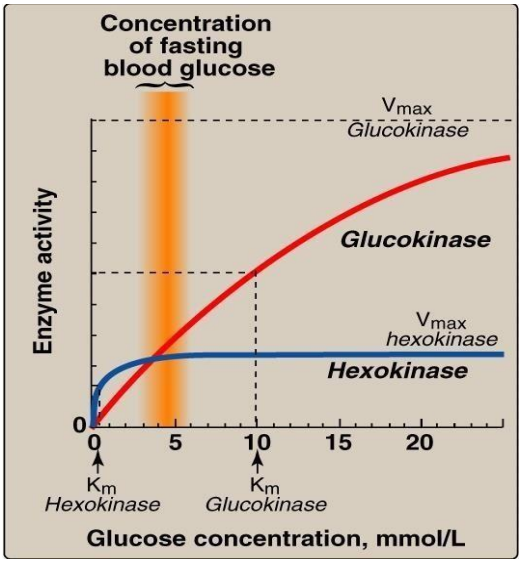
E- Protons (H+): it means that TCA cycle and oxidative phosphorylation are active and produce energy, in a way or another it indicates high energy levels. (Inhibitor)
Fructose-2,6-Bisphosphate has a tale that we will talk about soon …

2- Pyruvate kinase:
A- Fructose-1,6-Bisphosphate which is the product of the previous regulated step is an activator. (When the product of any step increases it will stimulate other steps to happen)
B- ATP is again an inhibitor that indicates a high energy level so there is no need to breakdown more glucose.
C- The last one is Alanine an amino acid which become pyruvate… here is an explanation darling …

When Alanine is metabolized it releases NH3 the amino group and becomes α keto acid this α keto acid of Alanine is our lovely pyruvate… I guess that you reach the information that Alanine is the source of pyruvate... U are awesome!!!

And since we have the end product why to breakdown more glucose? That's why Alanine works as inhibitor.

GLUCOKINASE AND HEXOKINASE ACTIVITY:



And now let's talk about glucokinase and hexokinase focusing on their kinetics.

Hexokinase works in all cells all the time at low concentration of glucose so its Km is very small with high affinity of glucose … but why?...

To catch (يلقط) any glucose presented under these conditions, whereas Glucokinase is activated by high amounts of glucose for example after a meal … so its stimulated not self-activated all the time, it is expressed by hepatocytes in the liver and gets activated under high concentrations of glucose … our gorgeous doctor called glucokinase system (راعي الفزعة او سستم الفزعات)

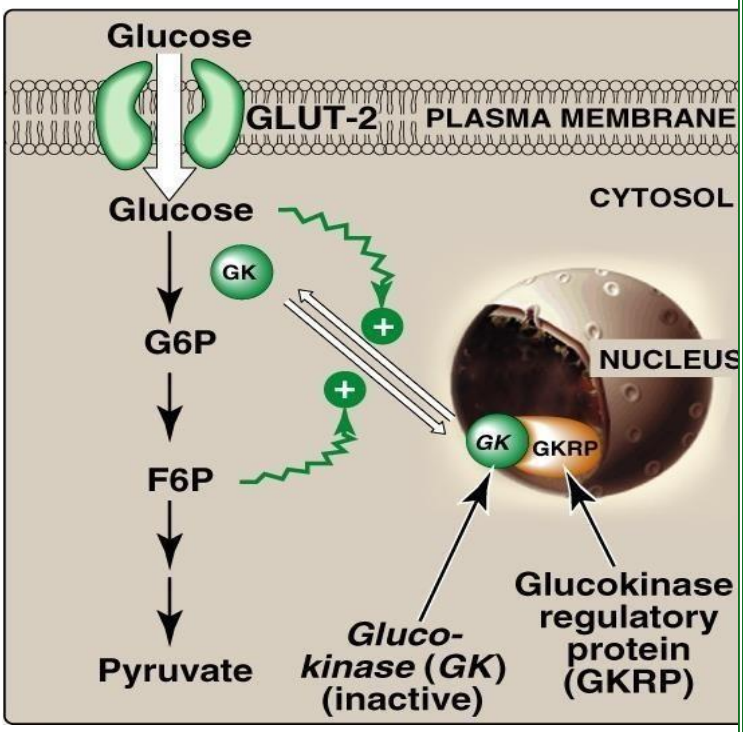
remember that Km is half of V max.

Km value of Glucokinase is much larger than that of Hexokinase and that's why its affinity for glucose is less it wont bind glucose unless its concentration is high.

The orange zone here indicates the level of sugar under fasting conditions (fasting blood sugar), if the level of glucose falls down this level this will cause loss of consciousness. تمام؟؟؟

at the level of fasting hexokinase is working at Vmax but glucokinase V is much lower than Vmax, it doesn't even reach 1/4 Vmax.

REGULATION OF GLUCOKINASE:



To regulate the activity of glucokinase in the cell it is bounded to a regulatory protein (GKRP) Glucokinase Regulatory Protein which works in sequestering glucokinase in the nucleus

(زي كأنه بينفي الجلوكوكاينيز و برميه بالنواة بعيدا عن مكان عمله)

Because glucokinase cannot function in the nucleus it only functions in the cytosol so as soon as it is in the nucleus it is inactive, so when it becomes activated?

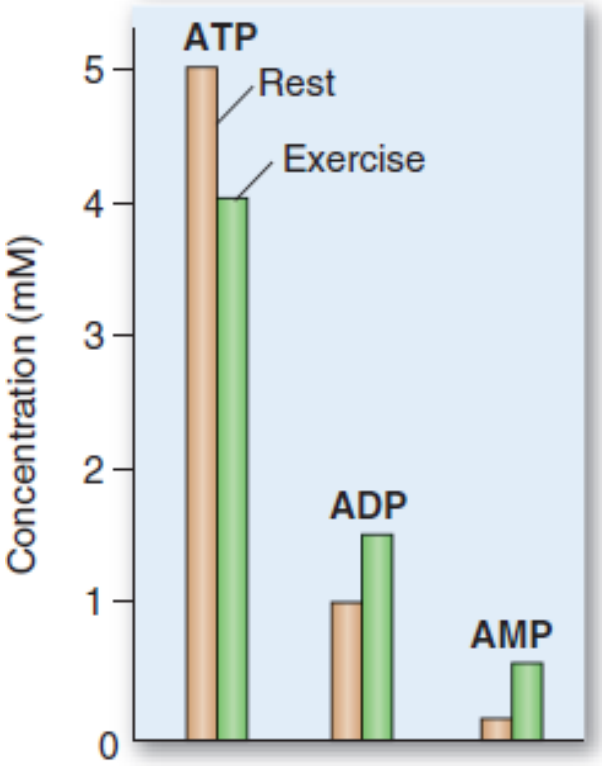
When Glucose concentration is high after glucose enter the cell through GLUTs.

The presence of glucose will activate the untie of this binding so the glucokinase can be released into the cytosol and it can act on glucose to phosphorylate it to Glucose-6-phosphate and then continue in glycolysis.

When Fructose-6-phosphate is generated due to the degradation of glucose this would activate the sequestration of glucokinase back to the nucleus as a result GK will return back to nucleus binds to GKRP and become inactive.

وكأنه بيقول للإنزيم ما فش داعي تضل تكسر جلوكوز كتير مدام صار عندك كمية كبيرة من الفركتوز 6 فوسفات

REGULATION BY ATP AD AMP:



In this slide the doctor is shoeing us the ATP, ADP and AMP under rest and exercise conditions, which explains the regulation of glycolysis by ATP and AMP.

We already said that ATP and AMP appear in more than step affecting more than enzyme either as activators or as inhibitors.

ATP level increases under rest because we are not consuming it and decreases under exercise because we are consuming it.

In the other hand, ADP level decreases under rest when I am not consuming ATP and increases under exercise because we are hydrolyzing ATP to ADP to get energy.

I guess that u have realized with your luscious eyes our dear that AMP follows ADP so they are the same.

And that's how they indicate the energy state inside the cell and how this would affect the activity of our ugly lovely GLYCOLYSIS.

Look over this equation sweety….

ADP + ADP ⟶ ATP + AMP

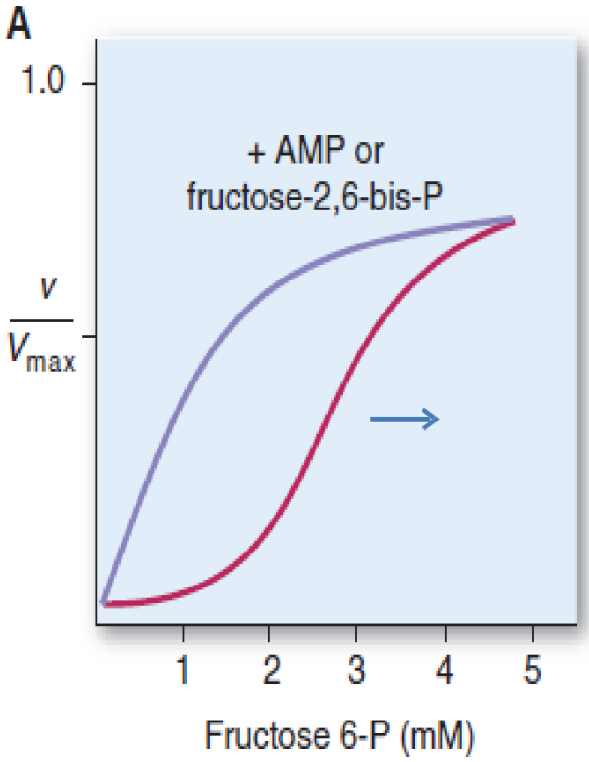
REGULATION OF PFK BY FRUCTOSE- 2,6-BISPHOSPHATE :

And now lets talk about the second enzyme which is phosphofructokinase isn't its name glum!!

We said that AMP is an activator.

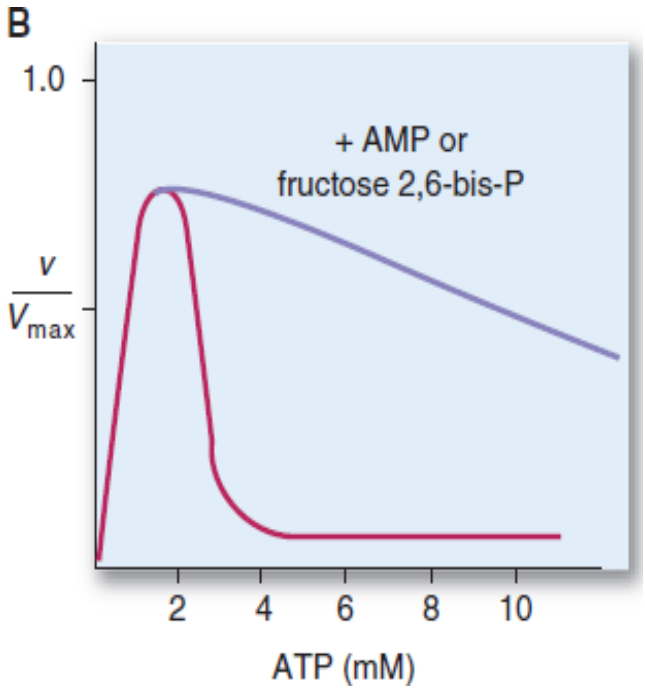
The pinkish red curve is the regular curve without any effectors neither activators nor inhibitors the conversion of F-6-P to F-2,6-bis-P.

When we add AMP or F-2,6-bis-P (activators) V is increased notice that we didn't increase Vmax what happened is that we shift the curve to the left which means that we can reach Vmax earlier at a lower concentration of the substrate which is F-6-P.



HOW ABOUT THE OTHER SUBSTRATE OF PFK ?

PFK brings phosphate from ATP which is a substrate during this reaction, look what happens in response to changes in ATP concentration alone without any other effectors.



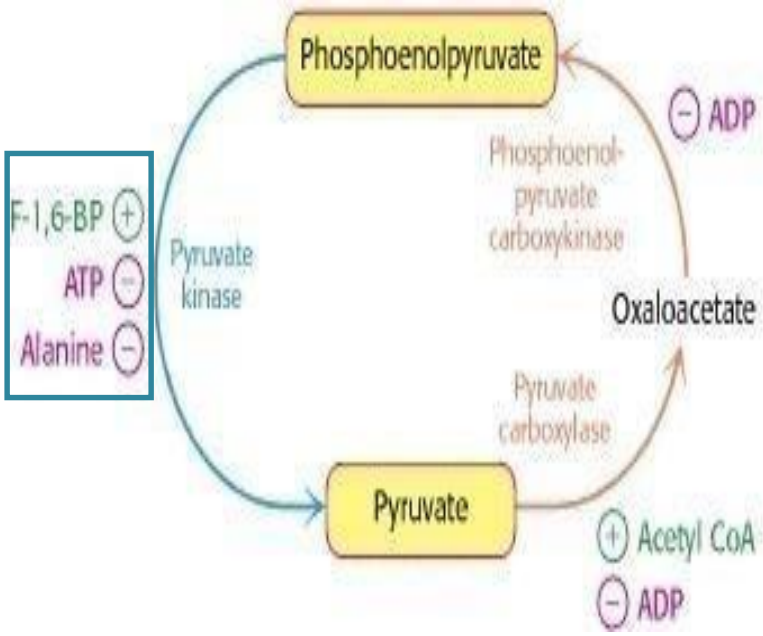
When ATP concentration increases firstly the velocity increases then it decreases so it is not the indicator because it is affected by the other substrate which is F-6-P, so if we don't have F-6-P even if we have a lot of ATP but we don't have anything to phosphorylate and that's why the curve goes down again.

When AMP or F-2,6-bis-P are presented the curve is going to go doen again but slower which give the enzyme better chance to work and to use a

higher concentration of the substrate… so here we have 2 curves for the same reaction but in relation or regard to different substrates.

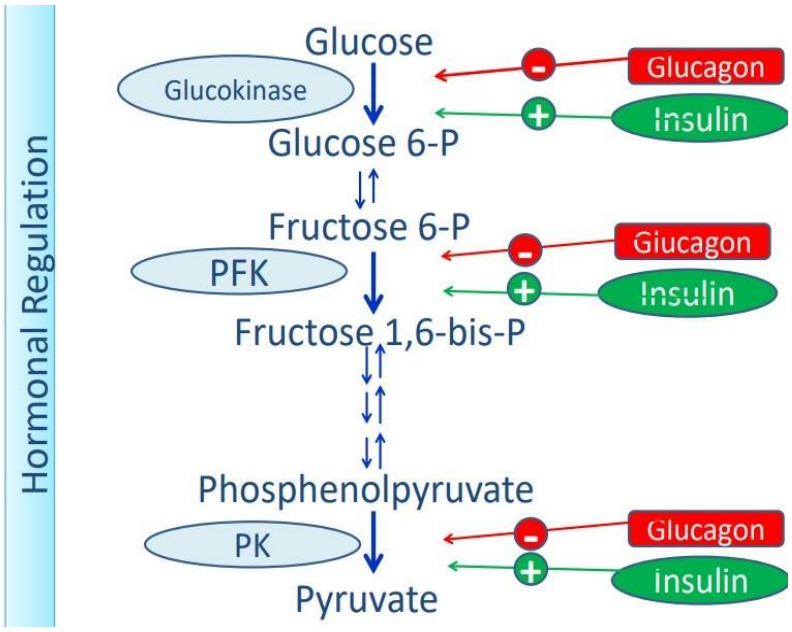
REGULATION OF PYRUVATE KINASE:

we already said that F-1,6-BP activates it while ATP and Alanine inhibit it.



HORMONAL REGULATION

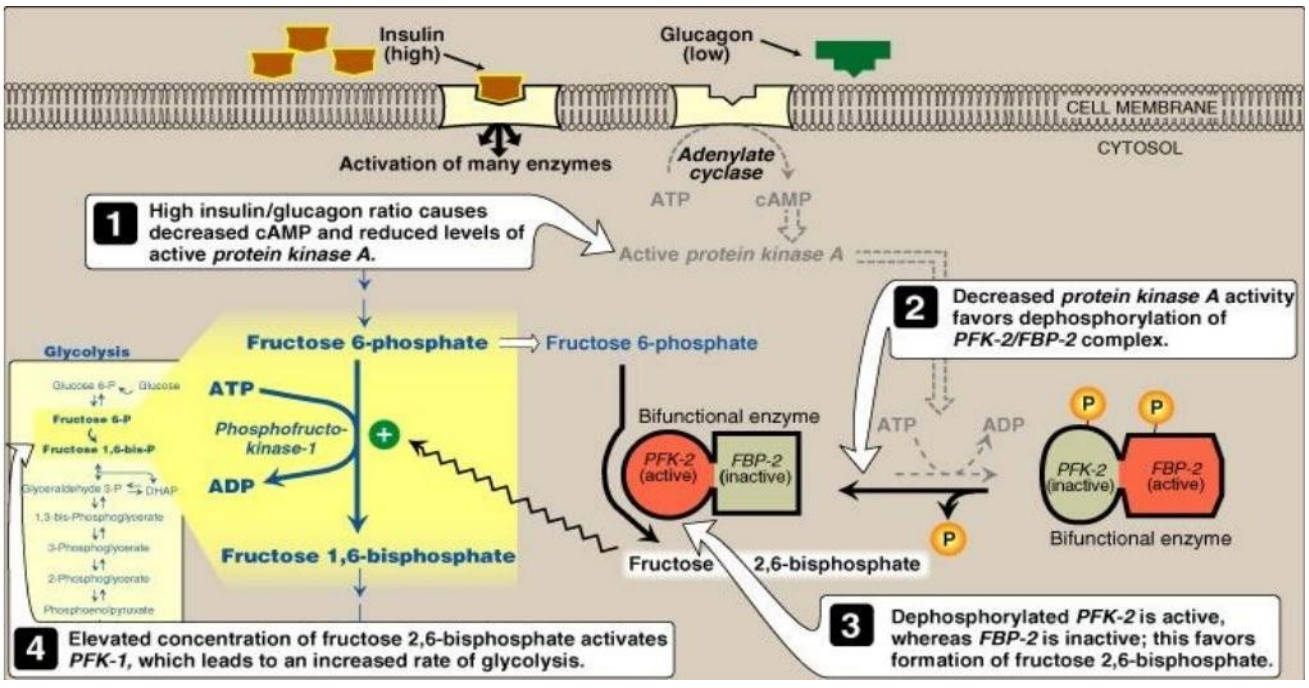
Discussing hormones now, we should re-emphasize that glycolysis is active at high glucose conc. **where Insulin is predominantly active, note how Insulin activates all the three irreversible steps. On the other hand, glucagon inhibits all of them.** THEY MUST OPPOSE EACH OTHER IN THEIR MECHANISM OF ACTION.



- The insulin is secreted when there is a highconc of glucose at **well- fed state**, while theglucagon is released at **fasting condition**. Note that both hormones are secreted separately at different time.

- They actually don't directly bind to the enzymes regulating the process, rather they work as a part of a long signaling pathway that activates different downstream target molecules, one of them being a protein kinase that phosphorylates an enzyme switching it 'on or off.

HORMONAL REGULATION OF PHOSOHOFRUCTOKINASE



NOTE: Insulin receptors are enzyme-linked receptors (RTKs or receptor tyrosine kinases) while glucagon performs its action through G-protein coupled receptor (GPCR).

- The occurring situation in the figure is the high presence of Insulin compared with lower glucagon. Provided that glucagon conc. is low, activation of GPCR &Adenylate cyclase won't be initiated, thus cAMP won't get activated in order to stimulate downstream target proteins, including protein kinase (e.x: PKA&PKC). Note that faint pathway drawn out of glucagon.

- One of the prominent targets is the enzyme complex (bifunctional enzyme) consisting of PFK-2 (phosphofructokinase-2) & FBP-2(Fructose-bisphosphatase2), which are completely opposite in terms of their function, with one phosphorylates and the other dephosphorylates. Thus, they mustn't be active at the same time.

- If the glucagon had been present, and the pathway proceeded, this complex would have been phosphorylated (by upstream proteins in the cascade, e.g: PKA), inactivating the 'kinase' part while turning on the 'phosphatase' one, which is fine and logical. Here glucagon pathway is locked, so the enzyme will be dephosphorylated, with kinase being active and phosphatase inactive.

- The substrate of these 2 enzymes (PFK-2 & FBP-2) is Fructose-2,6-bisphosphate, one of the allosteric activators of PFK-1 that changes its conformation preventing other inhibitors from binding (e.x: H, citrate).

  - High insulin⟶ PFK-2 active (**dephosphorylated**)⟶Fructose-2,6-bisphosphate increase ⟶ glycolysis activation.

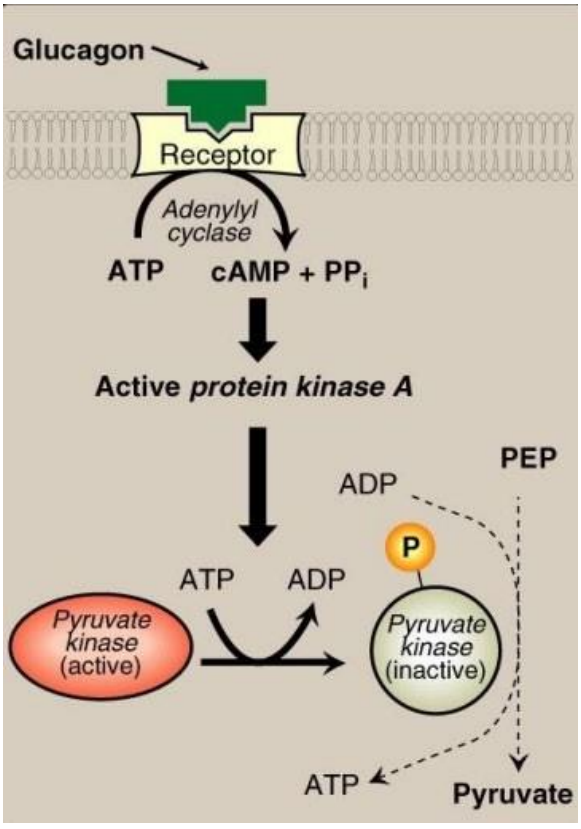
  - High glucagon ⟶ FBP-2 part is active (**phosphorylated**) ⟶ less Fructose-2,6- bisphosphate ⟶ deactivating glycolysis.

- Don't forget that the bifunctional enzyme responsible for the aforementioned situation (activating PFK-1 as well as glycolysis) is the dephosphorylated form, which results from a low conc. of Glucagon, and high conc of Insulin (increasing glucose levels).

- To sum up, when Insulin is present in low conc. (e.g: low sugar), glucagon is the manipulator now (the faint pathway previously discussed will take place). GPCR is activated followed by cAMP and the subsequent protein kinases (PKA&PKC), the latter will phosphorylate the bifunctional enzyme (the 'kinase' part is inactive, the 'phosphatase' is active-opposing the first situation). Now, the active phosphatase is going to remove the additional phosphate of Fructose-2,6-bisphosphate, returning it into Fructose-6- phosphate. As a result, the activator of PFK-1 is absent, which will inhibit the enzyme and inevitably the glycolytic pathway.

HORMONAL REGULATION OF PYRUVATE KINASE

- Glucagon regulatory effect doesn't manifest only in modulating PFK-1 function by the bifunctional enzyme complex, yet there are many other target proteins, activated by GPCR, cAMP and the downstream protein kinases (such as PKA).



- The target of interest now is Pyruvate kinase, the enzyme responsible for pyruvate production from phosphoenolpyruvate (the 10th step).

- When Pyruvate kinase gets phosphorylated by PKA, it switches to the INACTIVE STATE. This does perfectly make sense because glucagon naturally inhibits glycolysis so working on the enzyme responsible for such a significant step that outlines glycolytic progression will definitely inhibit it.

CLINICAL HINT: PYRUVATE KINASE DEFICIENCY

The most common among glycolytic enzyme deficiencies. (due to genetic mutations)

RBCs are affected (because it is mainly dependent on glycolysis unlike other cells that also undergo Krebs cycle)

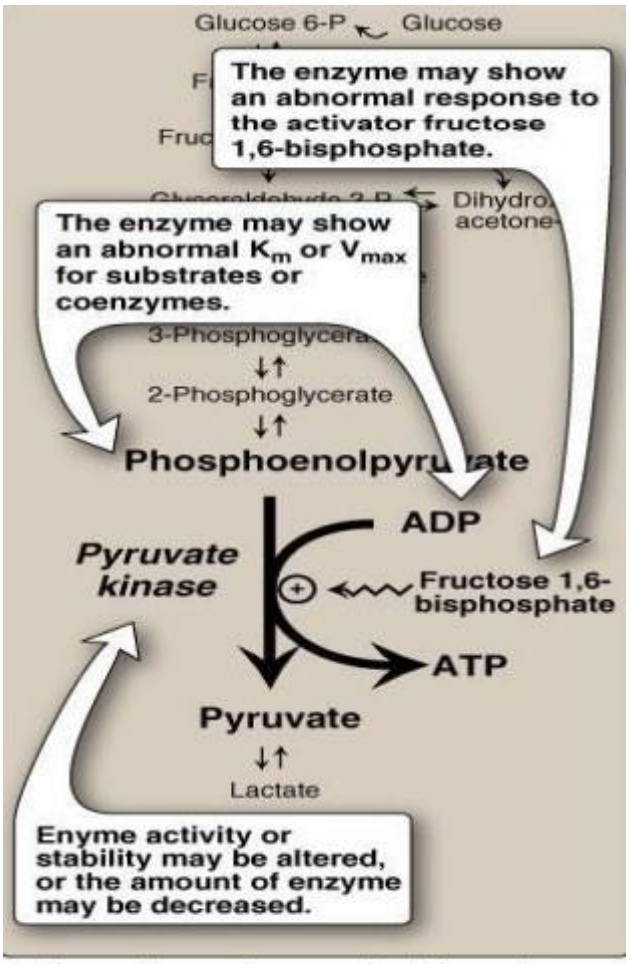
RBC lacks mitochondria so there is no Krebs cycle in RBC.

RBC losses its flexibility due to malfunctioning of Na+ - K+ Pumps due to the reduced amount of ATP.

Accordingly, RBCs energy will be very low, therefore, Na+ - K+ Pumps will be deficient so the shape of RBCs will not be maintained (losing of shape leads to losing of function) so RBCs will die (Hemolysis).

Side note: RBC has biconcave shape.

To sum up,without pyruvate kinase deficiency RBCs have a zero net energy yield from glycolysis. HAVING PYRUVATE KINASE DEFICIENCY, THIS ZERO BECOMES '-2'.



Alterations observed with various mutant forms of pyruvate kinase

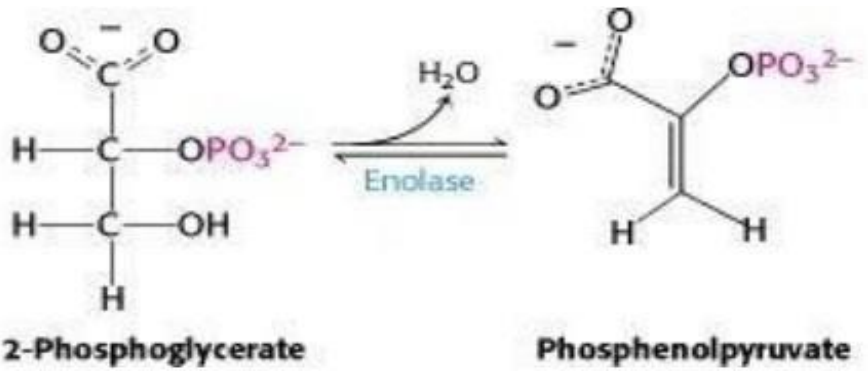
- **Mild to severe chronic hemolytic anemia** (depends on the kind and severity of the mutation)
- **ATP is needed for Na+/K+ pump ⟶ maintain the flexible shape of the cell.**
- **Low ATP ⟶ premature death of RBC**
- **Abnormal enzyme; mostly altered kinetic properties**

INORGANIC INHIBITORS OF GLYCOLYSIS
FLUORIDE

It is a regulatory mechanism from outside the body rather than inside such as Fluoride .

- **Fluoride inhibits Enolase**

- **Fluoridated water ⟶ bacterial enolase ⟶ Prevention of Dental Carries.**



We can find Fluoride in toothbast and in water .

بعد دراسات عديدة في الطب الوقائي وجد أن إضافة الفلورايد على معجون الأسنان أو الماء بكميات ضئيلة جدا (جزء من مليون) تخفف نسبة التسوس على مستوى العالم
الكالسيوم يساعد في نمو الأسنان ولكنه غير فعال بإزالة التسوس من الأسنان كالفلورايد
معجون الأسنان للأطفال لا يحتوي على فلورايد لأنه في حالتهم بلعه سيصلهم تركيز عال من الفلورايد فيصابوا بالتفلور

Fluorosis التفلور

A relatively high dose of fluoride has an opposite effect (it weakens the structure of teeth)

ARSENIC POISONING

Another external inhibitor is Arsenic

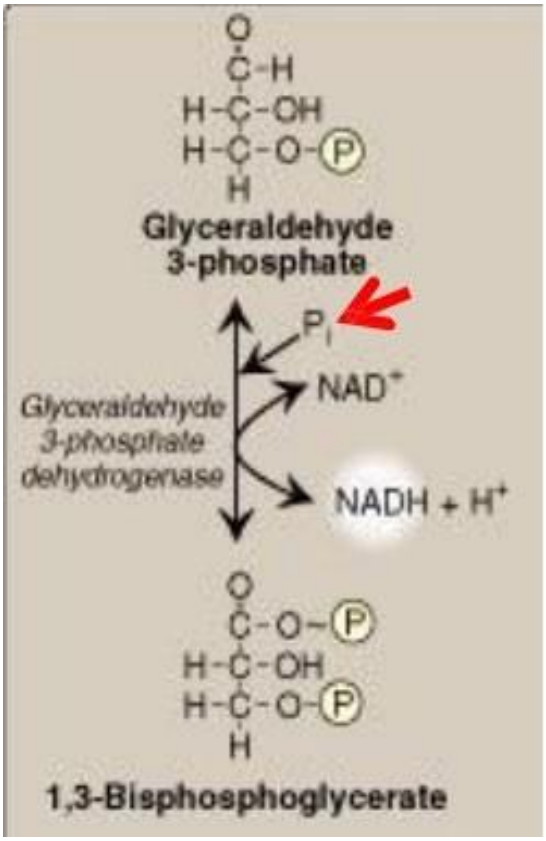
Arsenic is a transition metal so it has two ionizational states (Pentavalent Arsenic and Trivalent Arsenic).

- **Pentavalent Arsenic (Arsenate) competes with phosphate as as a substrate for GA3PDH**

  ↓ **ATP synthesis.**

Arsenate works on the beginning of second phase of glycolysis especially on the step which converts Glyceraldehyde-3-Phosphate to 1-3-Bisphosphoglycerate

Arsenate is going to compete with inorganic phosphate (which is added to carbon number 1 of Glyceraldehyde-3-Phosphate), after this competition there will be no phosphate that can be used to generate ATP in the next step, so energy will be reduced.



- **Trivalent Arsenic (Arsenite) Forms stable complex with -SH of lipoic acid.**

  ↓ **Pyruvate Dehydrogenase**

  ↓ **α ketoglutarate Dehydrogenase**

  → **Neurological disturbances…….DEATH**

- **Arsenite** acts on another enzyme which is Pyruvate Dehydrogenase (which converts Pyruvate to Acetyl CoA).
- **Arsenite** forms a complex with lipoic acid (as we remember Lipoic acid is one of the coenzymes that is used for E2).
- **Arsenite** is much more dangerous than Arsenate.

- **Arsenate** reduces ATP although it can undergo Glycolysis WHILE Arsenite reduces the activity of Pyruvate Dehydrogenase so Acetyl CoA content will be reduced then Krebs Cycle will be affected, more and more leading to severly reduced energy (it may causes death)

- To sum up, **Arsenite** affects Lipoic acid so Krebs will be affected. Lipoic acid is present in both alpha-Ketogluterate and Pyruvate dehydrogenase, so they both get affected by Arsenite poisoning.