DOCTOR 2020 | JU

METABOLISM

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Quick recap:

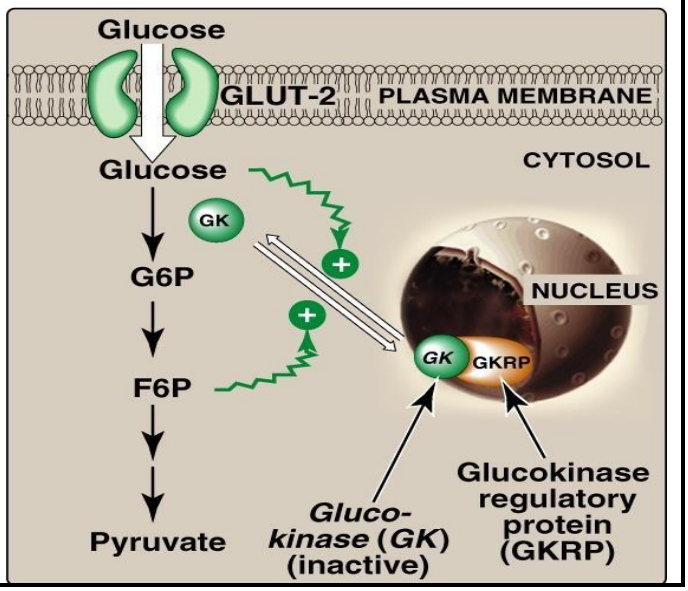
Last time we were talking about regulators of the glycolytic pathway, emphasizing that regulation happens at the three irreversible steps:

- **STEP 1: Conversion of glucose to glucose-6-phosphate.**
- **STEP 3: Conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, by phosphofructokinase 1 (we'll be talking about PFK-2 shortly)**
  Phosphofructokinase is activated by fructose-2,6-bisphosphate (produced by PFK-2) and AMP and inhibited by ATP, citrate and H^+. Remember:
  - **ATP is energy currency and the presence of ATP is a marker of high energy state. However, AMP is a marker of low energy state.**
  - **Citrate is the key molecule in Krebs cycle (Citric acid cycle), so citrate abundance indicates that Krebs cycle is highly active and produces high energy which result in inhibition of glycolysis because we have enough energy from Krebs cycle.**
  - **NADH and H+ are proportional (NAD+ + H2 → NADH + H+), so high proton concentration indicates that there is high NADH concentration which enable us to generate energy by oxidative phosphorylation, so there is no need to glycolysis.**
- **STEP 10: Phosphoenolpyruvate to pyruvate by pyruvate kinase**
  Pyruvate kinase is activated by fructose-1,6-bisphosphate (the product of the previous step) and inhibited by ATP and Alanine (Alanine is a source of pyruvate (it can generate pyruvate by itself), so there is no need to glycolysis)

Furthermore, we've differentiated between hexokinase and glucokinase in the terms of enzymology, that hexokinase much more sensitive to glucose presence in the cell, being active and functional all the time with lower Vmax. Whereas glucokinase is activated only at certain conditions (e.x: certain conc. of glucose) **from here we can start our lecture**

As we said above, glucokinase is turned on or off depending on certain conditions such as the presence of hormones, specifically INSULIN.

When Insulin conc. is high, this means that sugar is present at high conc., which will induce continuously active glycolysis. Accordingly, Insulin will bind to its specific receptor (RTK) activating it, then the latter will activate a downstream signal cascade of proteins, which will end up activating transcription factors that turn on the genome to finally produce certain proteins (e.x:GLUT-4).



Note: the doctor said that GLUT-2 present in the above figure should be GLUT-4

After that, GLUT-4 expression on the cell membrane will significantly increase, thus more uptake of the sugar occurs.

Let's get a step back to glucokinase in its inactive state (e.g: before Insulin secretion and glucose entry). Actually, glucokinase was sequestered to the nucleus, bound to a regulatory protein called Glucokinase regulatory protein (GKRP) that stabilizes it in the inactive state. Once we have high expression of glucose transporters following glucose entry to the cytosol, glucose levels will be increasing, activating the separation of glucokinase from its regulatory protein, hence its movement back to the cytosol, where it's normally active and functional.

BOTTOM LINE: Glucose is responsible for glucokinase activation, by inducing the separation from its regulatory protein (its inhibitor) then it's released to the cytosol, its place of function.

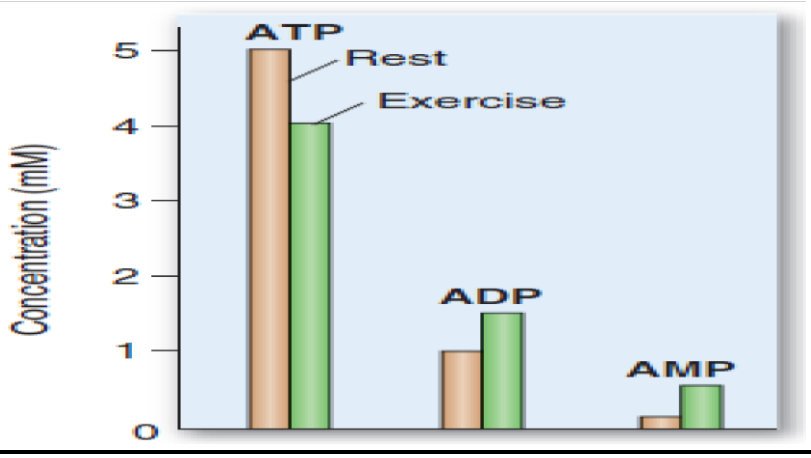
After activating the pathway, it will proceed to form glucose-6-phosphate then fructose-6-phosphate,etc, until glycolysis is finished. As a result of running these reactions several times, the intermediates will definitely be accumulating, strictly speaking about fructose-6-phosphate. The increasing conc. of fructose-6-phosphate will –at some point- inhibit glucokinase .HOW DOES THIS HAPPEN?

Note that green positive sign rising from fructose-6-phosphate towards glucokinase. WATCH OUT! It doesn't mean activating the enzyme,yet it resembles that F6P is activating the return of the enzyme to the nucleus, again bind to GKPR and become inactivated, that does make sense because glucokinase won't be active all the time.

BOTTOM LINE: Increasing conc. of F6P will inhibit glucokinase by sequestering it back to the nucleus where it binds again with its regulatory protein and becomes inactive.

We also discussed the role of ATP&AMP being regulators of glycolysis, considering ATP a marker of a high-energy state, thus it inhibits the pathway. In contrast to AMP, the marker of low-energy state in the cell acting as an activator.

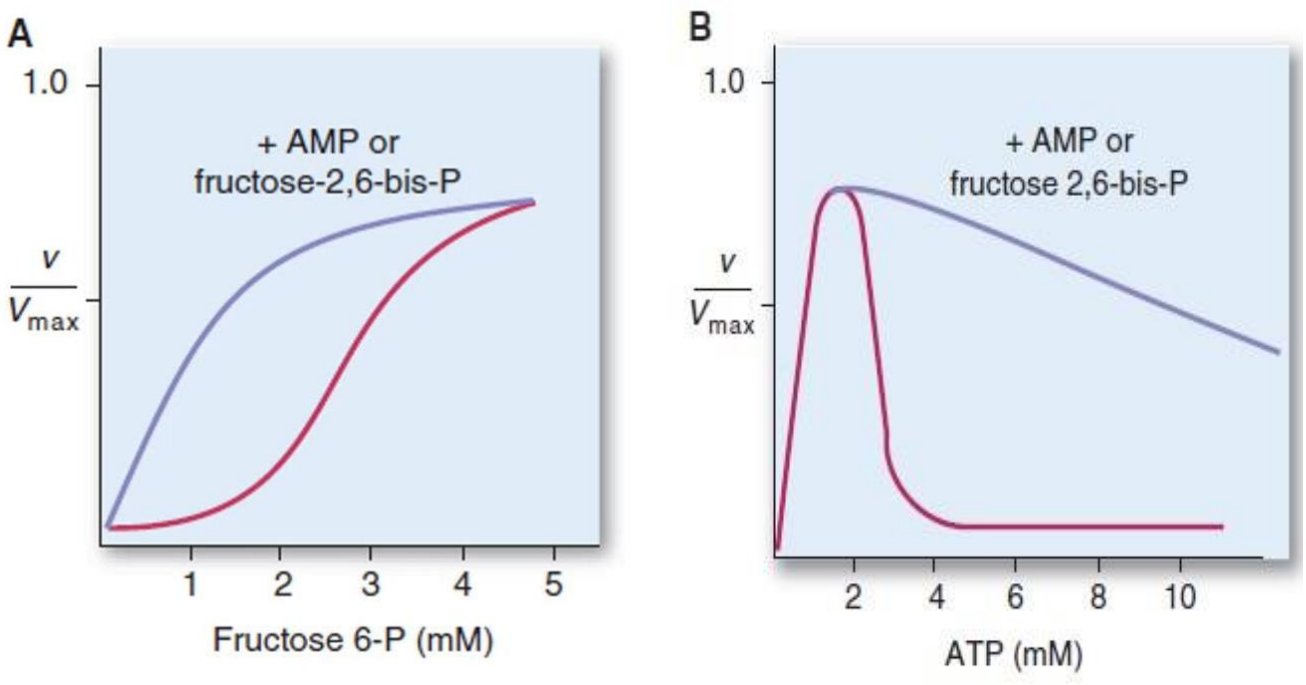
Under different conditions (resting state V.S exercise), note that ATP conc. is higher at resting state, compared with exercise in which the cell readily consumes it.



On the other hand, AMP&ADP conc. somehow adopt different manner, being elevated during exercise more than under resting conditions. Actually, this sounds logical, because they are the products of ATP hydrolysis, which is extensively used during exercise.

ADP+ADP ⇌ ATP+AMP

Regulation of PFK by fructose-2,6-bisphosphate



In the figure A, notice the pink curve that represents Fructose-6-phosphate normal reaction progress (without the presence of activators),it starts with a low conc. that is gradually increasing to reach a certain velocity .Now notice the difference after adding the allosteric activators(AMP&Fructose-2,6-bisphosphate) shown as the blue curve, the curve has become much more hyperbolic, needing less conc. to achieve the same desired velocity (The result is shifting the curve more to the left).

In terms of allosteric activation, AMP&Fructose-2,6-bisphosphate bind to their sites (not the active sites) inducing a conformational change in the enzyme structure to switch to the active form. At the same time, they inhibit the binding of inhibitors, such as citrate and protons.

Also note that we reached the same Vmax in both situations, with the sole difference being the conc. needed to achieve a certain intensity of the response.

How about the other substrate? As we know, phosphofructokinase phosphorylates F6P using ATP, so ATP is also another substrate of this enzyme.

After drawing a different graph (cuz we're dealing with a different substrate), the pink curve represents the substrate without any regulators. Note how fast it reached the peak(of response),and also how fast the response declined.

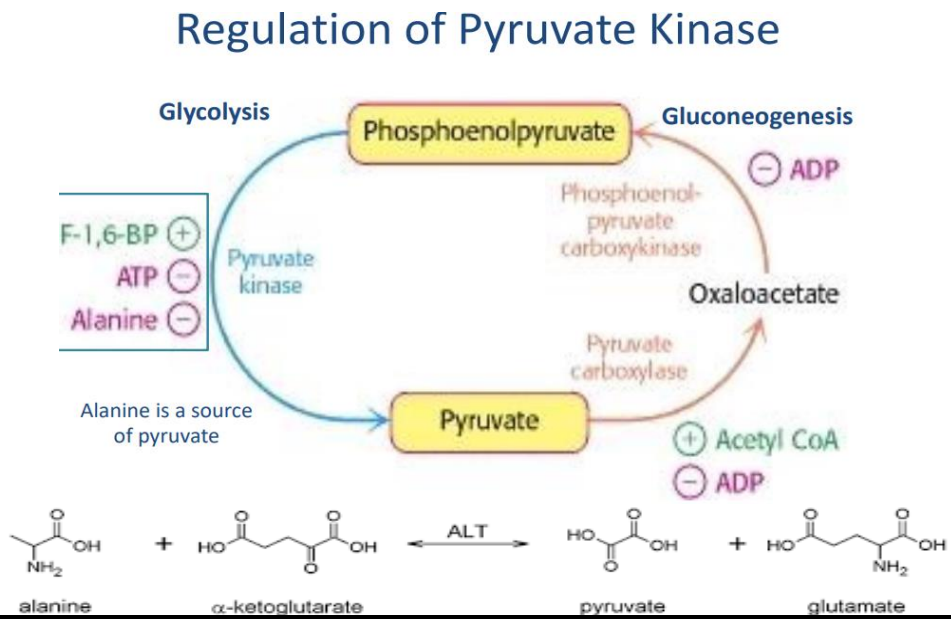
Now in the presence of activators (the blue curve), note that the response is gradually declining, but wait a minute! WHY DOES IT GO DOWN PRIMARILY?

Examining the previous figure, we see that F6P has reached Vmax in an ascending manner (rising), proving that it's the main substrate that guides the rxn process. As long as I have F6P,I use ATP. Therefore, what determines the productivity and effectiveness of the rxn is F6P, rather than ATP, and that's the main reason why ATP effect has been descending after rising for a period of time.

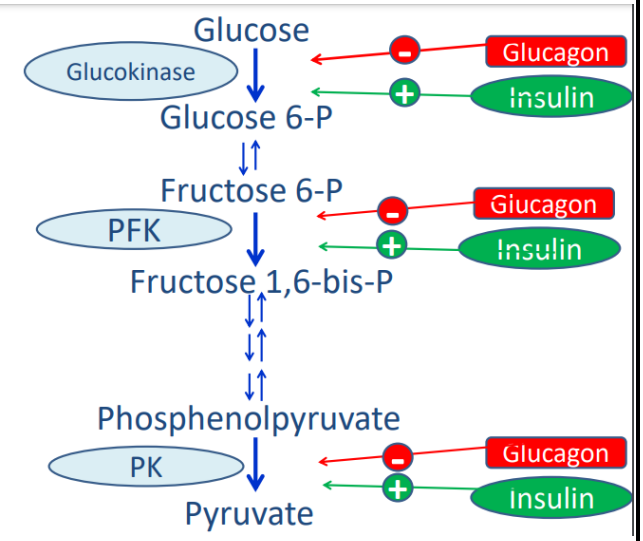
Even if I kept adding ATP, without F6P the rxn won't proceed. Thus, it will be of no significance.

The last irreversible step of glycolysis is phosphoenolpyruvate to pyruvate by pyruvate kinase,where Fructose-1,6-bisphosphate,the product of the previous step,activates this enzyme producing much more pyruvate.

Also we talked about ATP and Alanine and how do they act as inhibitors.That ATP is a marker of the high-energy state in the cell,...,while Alanine is the amino acid which when deaminated (transaminated) will produce the keto acid structure that's called pyruvate(A non-carbohydrate source of pyruvate) as shown in the figure below .Then when Alanine is abundantly present,this indicates a high conc. of pyruvate,inhibiting the reaction from further proceeding.



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.

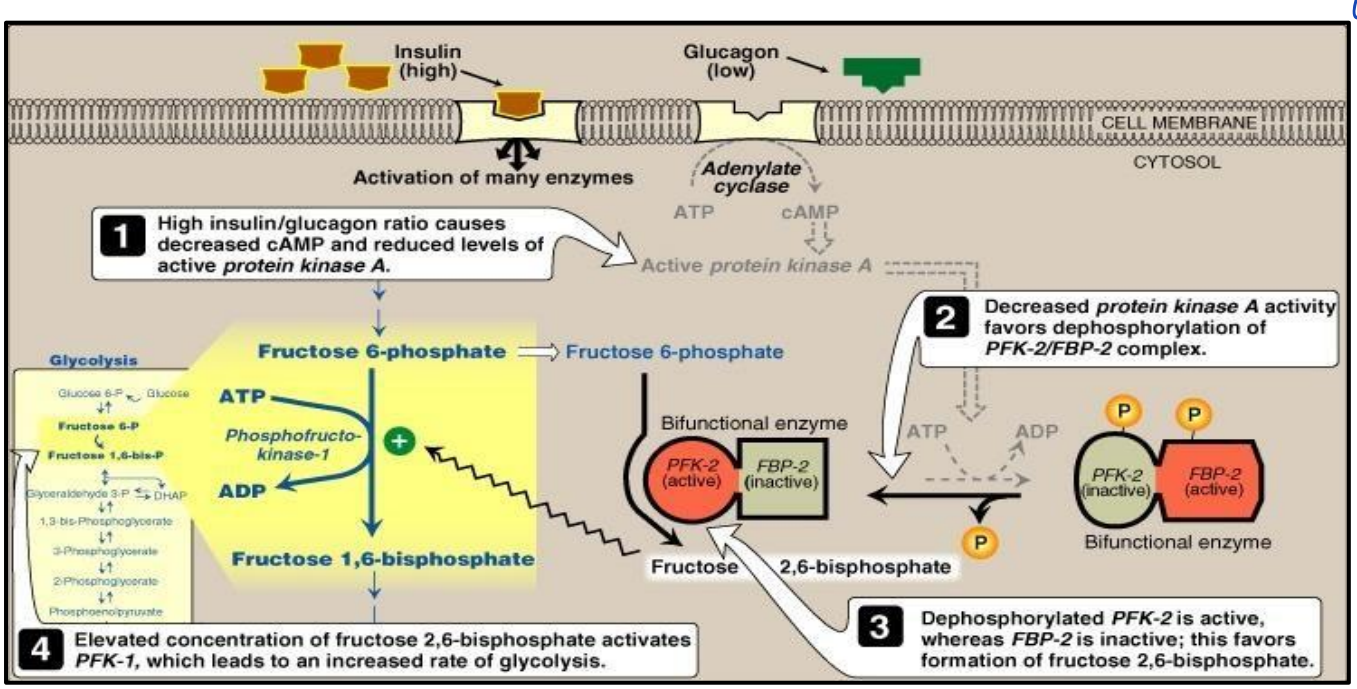


HOW DO THEY PRIMARILY WORK?

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'.

This figure below represents a cell surrounded by a CELL MEMBRANE that has receptors for Insulin & Glucagon. AGAIN THEY WON'T BE ACTIVE SIMULTANEOUSLY!!

Note that Insulin receptors are enzyme-linked receptors (RTKs or receptor tyrosine kinases) while glucacon 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-bisphosphatase 2), 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 ,…),

Insulin is high → Kinase part is active (dephosphorylated) → more Fructose-2,6-bisphosphate → activating glycolysis

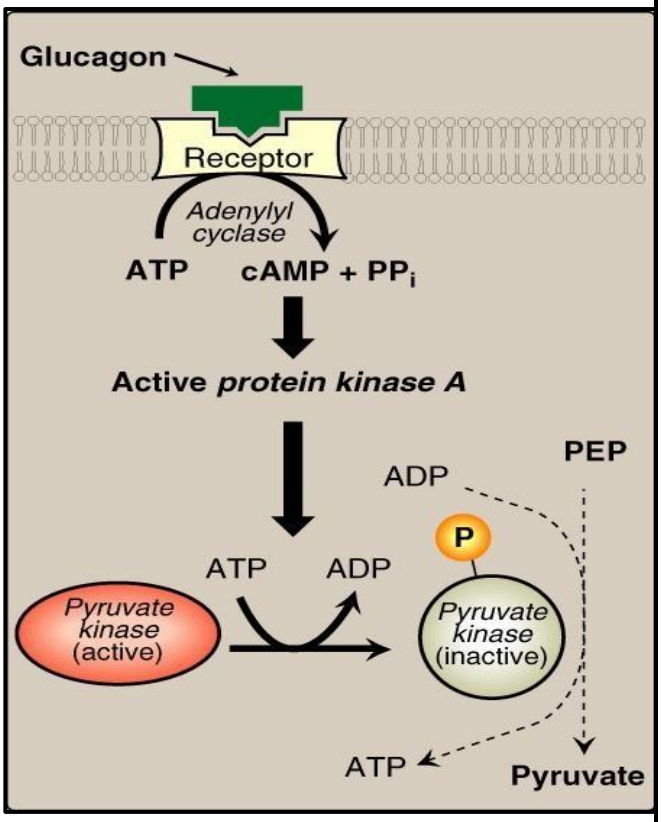
Glucagon is high → Phosphates part is active (phosphorylated) → less Fructose-2,6-bisphosphate → deactivating glycolysis

Note 1: the enzyme that converted Fructose-6-phosphate to Fructose-2,6-bisphosphate is PFK-2, which isn't a part of the glycolytic pathway.

Note 2: 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 highconc.of Insulin(increasing glucose levels).
Conversely, 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.

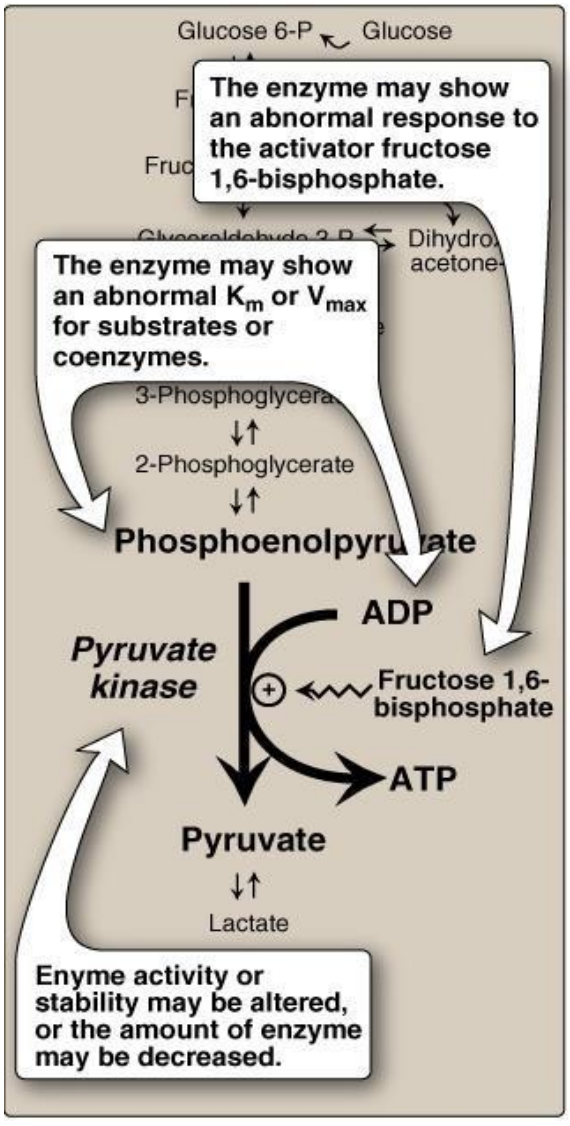
Glucagon regulatory effect doesn't manifest only in modulating PFK-1 function by the bifunctional enzyme complex, yet there are many other target proteins, activated by GPCR, cAMP and the downstream protein kinases (such as PKA). The target of interest now is **Pyruvate kinase** ,the enzyme responsible 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.
- It is acquired in a genetic manner (inherited).
- The clinical manifestation from mild to severe of the disease depending on the position affected on the gene that encodes pyruvate kinase (e.g: a certain position may be affected resulting in a 50% deficiency (moderate) or 80% deficiency (somewhat severe) or 20% deficiency (pretty mild).
- Targets mostly affected are RBCs. As they depend on glycolysis, which is not a significant source of energy for them, inhibiting the last step that produces pyruvate will result in the loss of another source of energy (2ATP resulting)
- Remember the first source of energy RBCs lack, which is the 2ATP produced during conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate.



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

Note: RBCs also generate energy by anaerobic respiration, through converting pyruvate into lactate, which will also be inhibited because of pyruvate deficiency marked in the last step (PEP to pyruvate).

And here RBCs will be aggressively working in order to compensate the sharp ATP depletion, though they don't need that much energy.

Despite this extensive work, they mostly won't be able to provide the needed energy supply. Therefore, RBCs are going to die prematurely (before 120 days), which results in hemolysis that will develop to hemolytic anemia.

Another bad consequence of ATP depletion is the severe effect on Na^+-K^+ ATP-dependent pumps, that function in not only maintaining membrane potential, but also maintain the special shape of RBCs (biconcave discs)

Let's break it down into two parts, in order to understand its significance. Firstly, biconcavity aids in the flexibility of RBCs, allowing them to smoothly pass through narrow blood vessels, in order to efficiently reach all tissues supplying them with their oxygen needs. This ofc won't occur if the whole cell structure was of the same thickness without concavities. Secondly discs, they contribute to the ease of movement of RBCs through the circulation.

So, whenever the shape is abnormal, the function is abnormal as well and this is really well observed in some disorders such as SICKLE CELL ANEMIA, where RBCs are 'sickle-like' shaped, losing biconcavity as well as efficient oxygen delivery to tissues.

Referring to the figure in the previous page, we can infer that the deficiency isn't the sole matter of concern of this disease, yet the enzyme itself gets abnormal, with substantially altered kinetic properties, represented by Km & Vmax becoming abnormal for substrates or coenzymes. Alterations can also affect the enzyme activity and stability, as well as the response to certain regulators (e.x: Fructose-1,6-bisphosphate).

LASTLY,WE'LL TALK ABOUT EXTERNAL INHIBITORS OF GLYCOLYSIS

External inhibitors (inorganic inhibitors): inhibitors of glycolysis that aren't present inside our body, that they are neither produced nor used in regulation and we might get exposed to.

- **Fluoride (inhibits enolase)**

When buying a toothpaste, you frequently notice a famous content, yes right! Fluoride

We use Fluoride specifically because it inhibits bacterial activity.

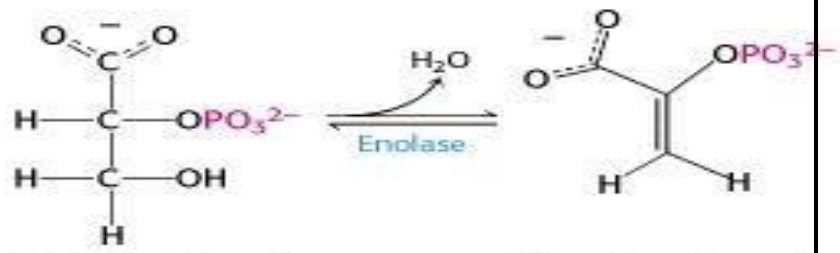
Normally, when anybody keeps eating sugar without brushing their teeth, it accumulates causing the formation of a layer of bacteria that make colonies. Thus, you hear it very much not to eat a big amount of sugar. However, the sugar bacteria benefits from in their synthetic processes is DEXTRAN, a very complicated and highly branched complexed polysaccharide. Hence when present abundantly, bacteria colonize utilizing this sugar as a source of energy.
As a side effect, unwanted products are produced including some acids, which will –with prolonged exposure- facilitate destroying teeth structure, leaving them necrotized and cavitated, despite that the outer layer of the tooth (enamel) is harder than the underlying bone, it can possibly decay with frequent exposure.
Regarding the previous scenario, when using Fluoride, you break this cycle.

Note: Regardless Fluoride is present or not, you're mechanically killing bacteria and removing the sugar residues.

When it comes to Fluoride can achieve a much better and long-lasting tooth integrity by 'soaking' your teeth with it for a couple of minutes after cleaning your teeth. By this, Fluoride adsorption occurs preserving its benefit.

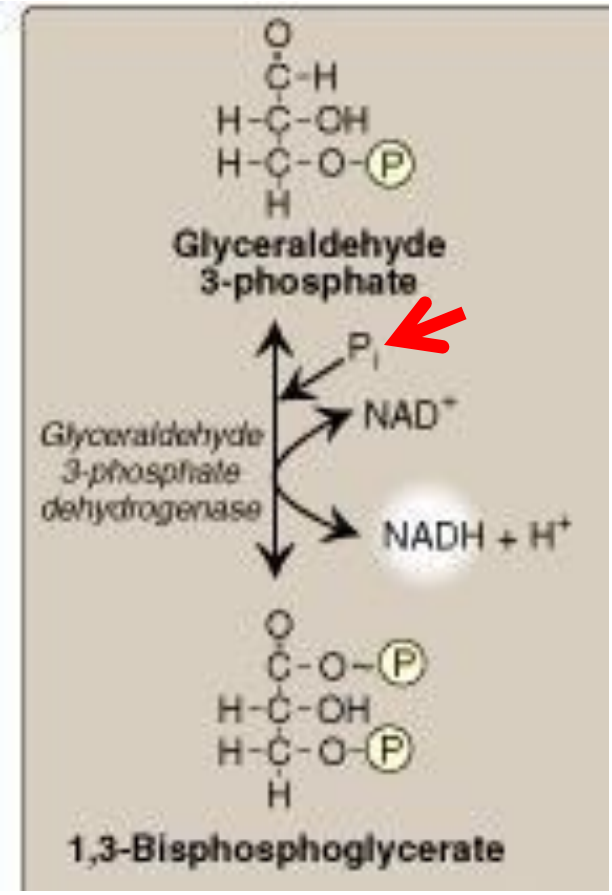
Let's relate to glycolysis.What Fluorine actually does is inhibition of glycolysis in bacteria, NOT OUR CELLS! Specifically, it inhibits bacterial enolase that converts 2-phosphoglycerate to phosphoenolpyruvate (step 9), thus no Acetyl
-CoA will be produced, no Krebs cycle,… (decrease in the bacteria energy that inhibits further division)
NOTE: PEP to Pyruvate (step 10) will be lost thus its energy yield is lost, same with the following Krebs energy-generating reactions, so a crucial amount of ATP will be wasted, leaving bacteria hard-hit.

Fluorinated water decreases bacterial enolase activity thus prevention of dental carries.



Arsenic Poisoning

- **Pentavalent Arsenic(Arsenate +5)**
  Competes with phosphate(Pi) as a substrate for Glyceraldehyde 3-phosphate dehydrogenase. Remember that this step(G3P to 1,3-bisphosphoglycerate) does not require ATP (we only used Pi for phosphorylation).So what happens here is competitive inhibition that prevents this step,preventing subsequent steps as well,leading to loss of energy and reduction of ATP formation.
- **Trivalent Arsenic (Arsenite +3)**
  It works on a reaction outside glycolysis, in which Pyruvate converts to Acetyl-CoA,by the action of Pyruvate dehydrogenase, a complex composed of multiple enzymes and coenzymes, one of the latter is Lipoic Acid. Arsenite forms a stable complex with the –SH group in LA , ceasing the rxn.



Notes about Arsenite mechanism of inhibition:

- **Note here that pyruvate production is normal, yet the problem is with Acetyl-CoA production, it will be inhibited, thus no Krebs cycle, etc. Eventually, this will cause neurological disturbances followed by death.**
- **Lipoic acid is present in both alpha ketogluterate (we took it in Krebs lectures) and Pyruvate dehydrogenase (this lecture), so they both get affected by Arsenite poisoning.**