Regulation of the Glycolytic Pathway

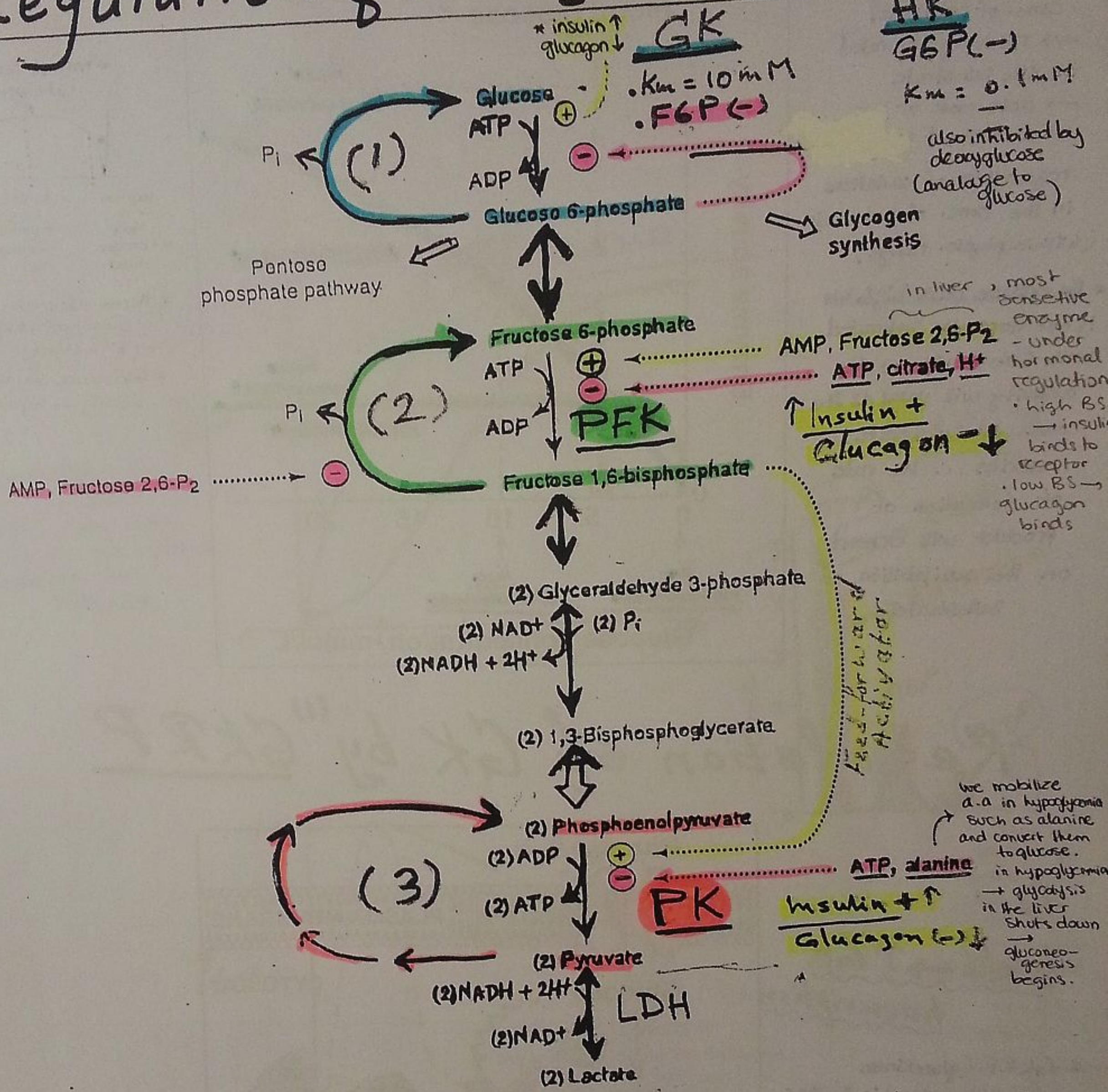


FIGURE 7.13
Important regulatory features of the glycolytic pathway.
Because of differences in isoenzyme distribution, not all tissues of the body have all of the regulatory mechanisms shown here.

* just to understand, own notes.

* note: * increased insulin → increases Glut-4 transporters → rate of uptake of glucose for glycolysis increases. it upregulates the transcription of Gk, Pfk and Pk levels.

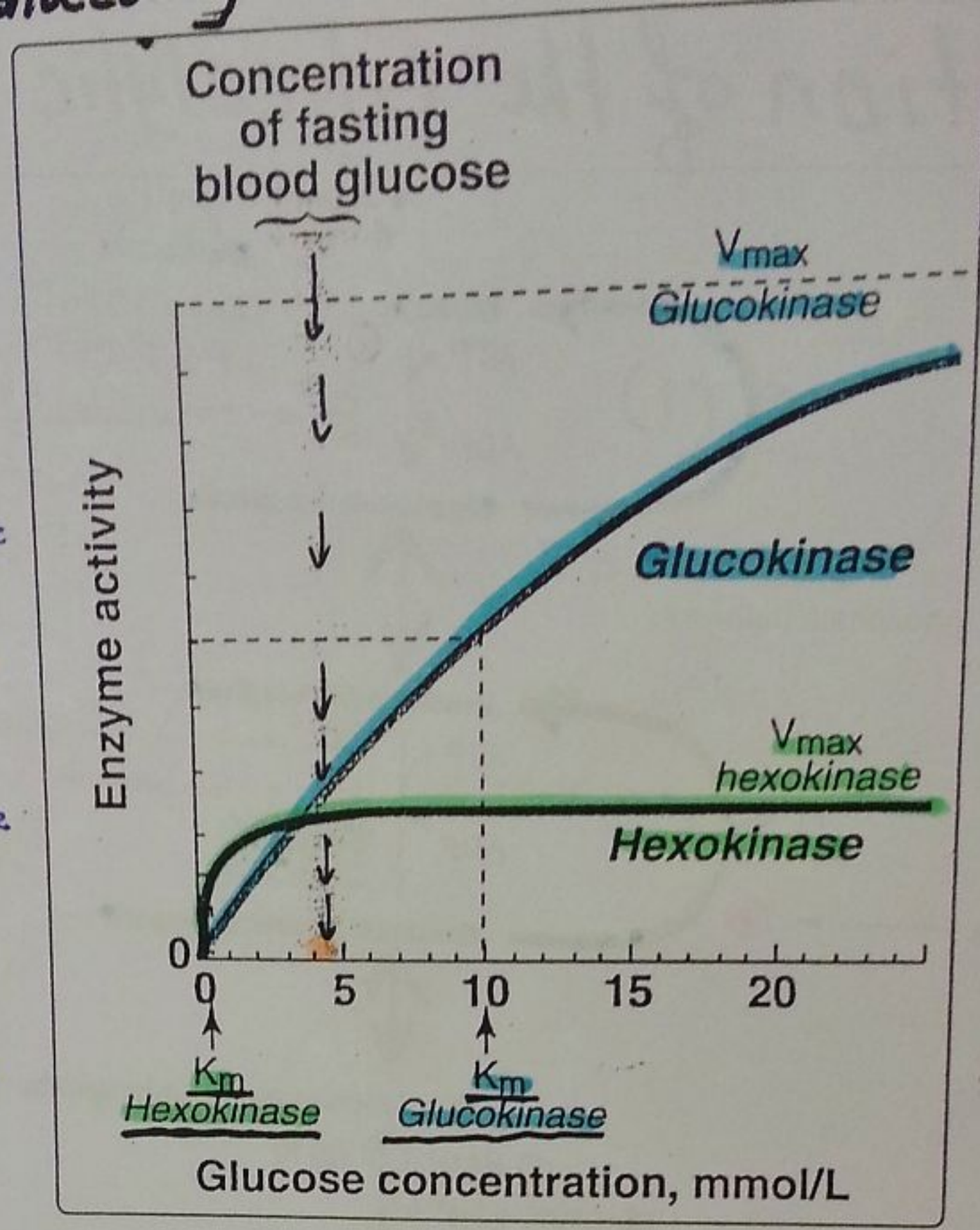
* glucagon has the opposite effect, its secretion is stimulated by low blood glucose levels, promotes breaking down of energy storage molecules when energy is needed. down regulates the transcription of those three hormones

• Vmax: rate of rxn when the enzyme is saturated with substrate = max rate of rxn
- the relationship between the rate of rxn and conc. of substrate depends on the affinity of the enzyme to its substrate, expressed as Km, an inverse measure of affinity.
* high Km → low affinity → requires a greater conc. of substrate to reach Vmax.

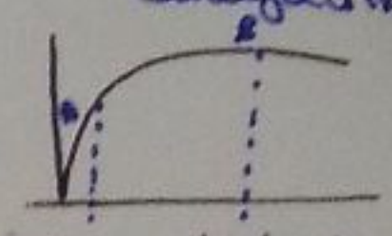
Effect of [illegible]

Notes:

* An enzyme with low Km (relative to physio. conc. of substrate)
  → normally saturated with substrate
  → acts more or less at a constant rate, regardless of variations in the conc. of substrate (within physio. range)
* An enzyme with high Km is not normally saturated with substrate → its activity will vary as the conc. of substrate varies ∴ the rate of formation of product will depend on the availability of substrate.



* normal enzyme catalyzed rxn:



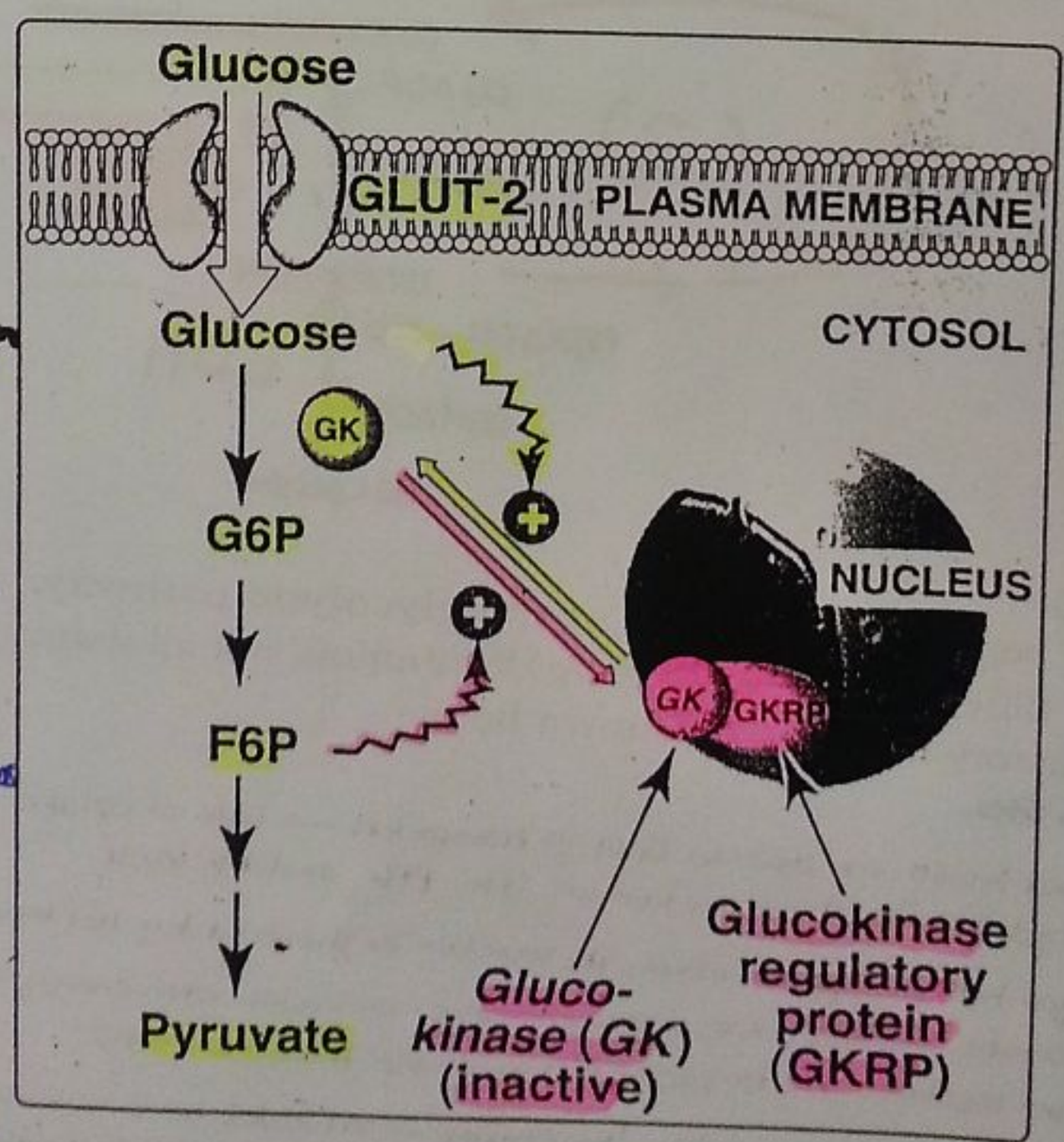
low sub. conc. → steep → enzyme unsat.

high sub. conc. → plateau → enzyme sat.

* Hexokinase reaches saturation quickly → high affinity
* Glucokinase has low affinity → higher Vmax

Regulation of GK by "GKRP

2) Insulin → ↑ GK transcription

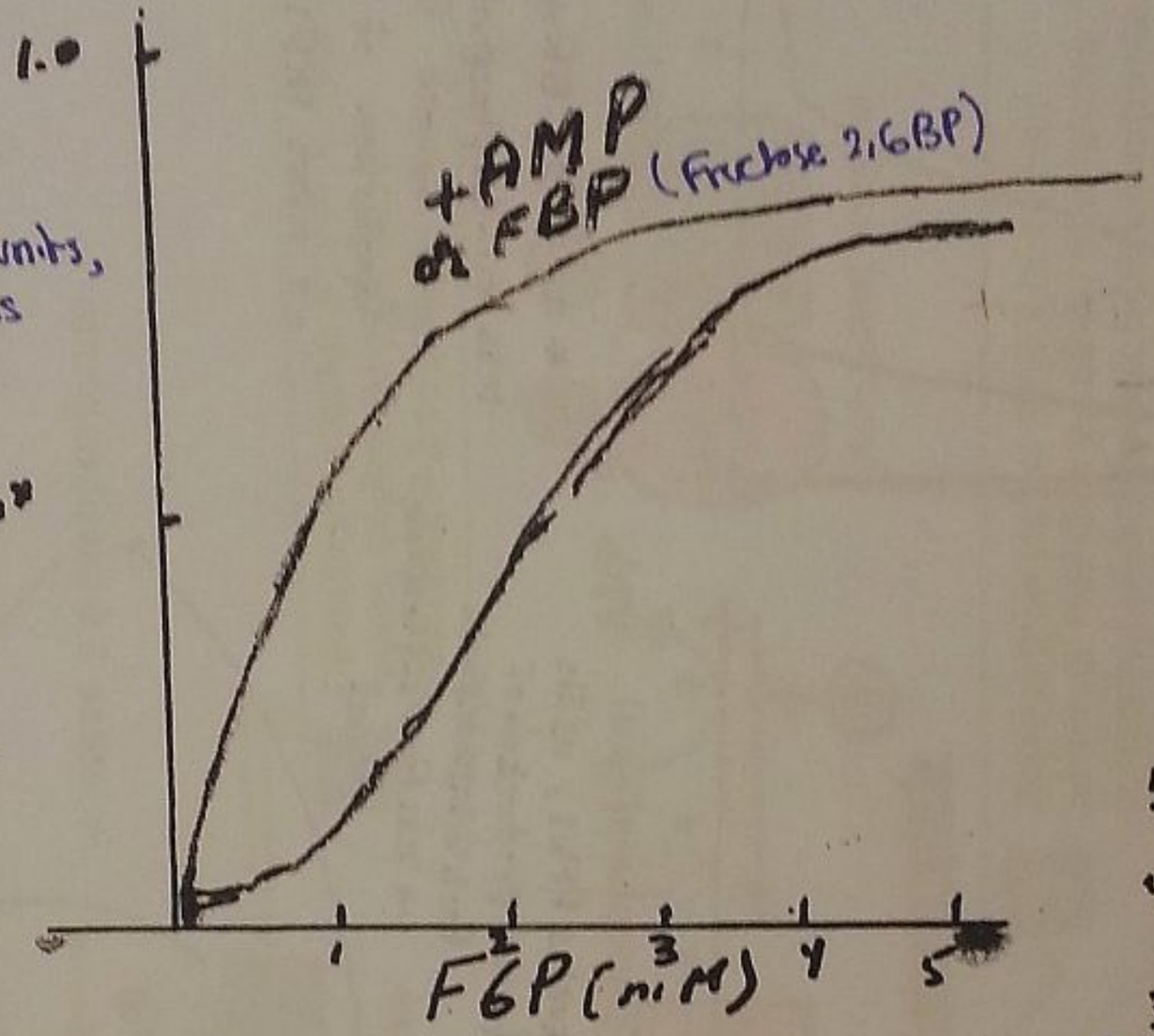


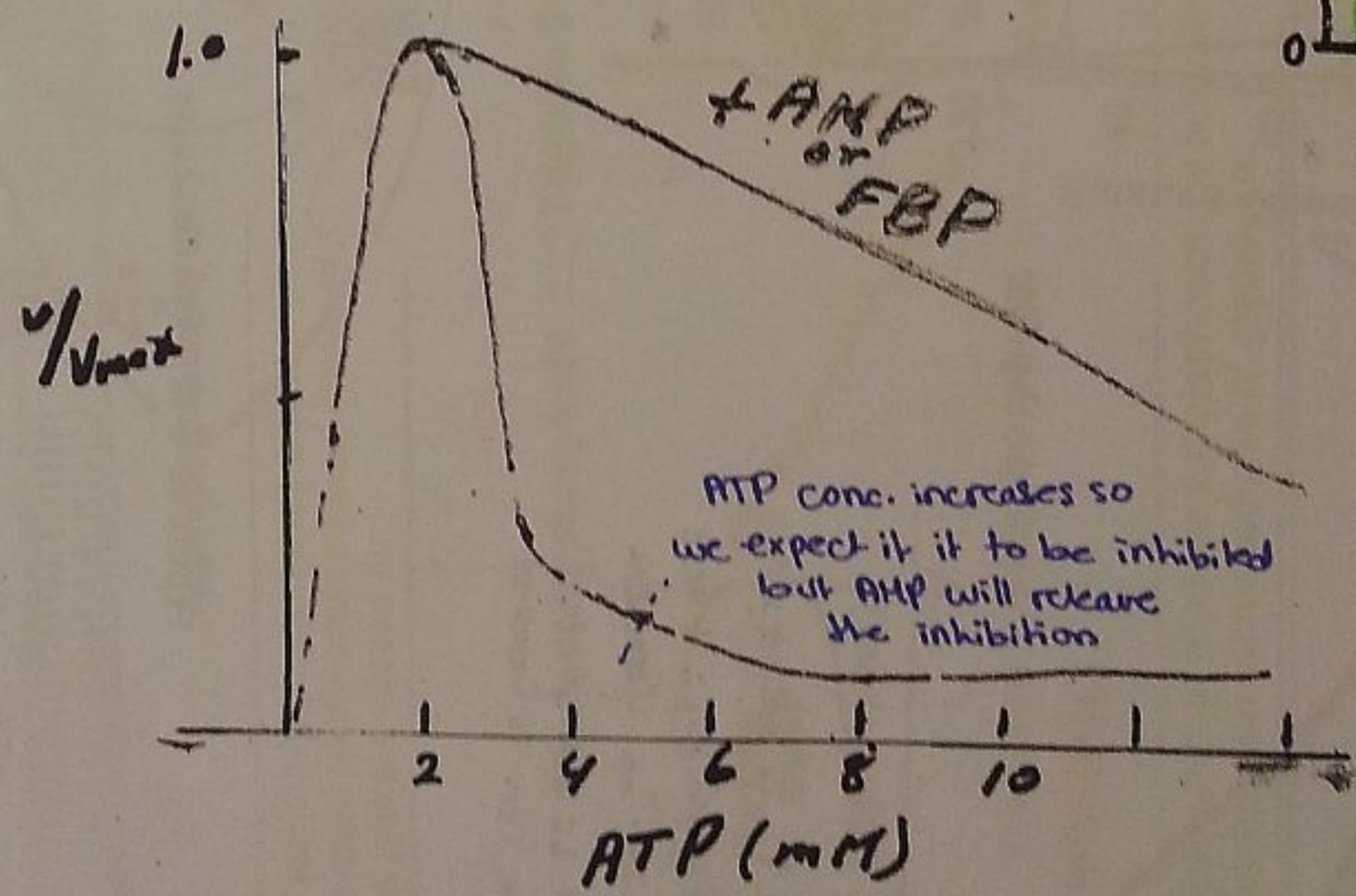
* GKRP (glucokinase regulatory protein) in the liver regulates activity of GK through reversible binding.
  - Fru 6 P available
  - GK → translocated into nucleus
  - GK binds tightly to GKRP
  - GK therefore is inactivated
  - when glucose levels in the blood (also in hepatocytes, due to GLUT-2) ↑
  - GK is released from GKRP
  - enzyme reenters cytosol
  - Glucose is phosphorylated → G6P.

Regulation of PFK by AMP and ATP

* PFK consist of multisubunits, it shows sigmoidal kinetics with F6P.

* Sigmoidality can be abolished and the curve shifts to the left by curves activators like AMP, FBP.



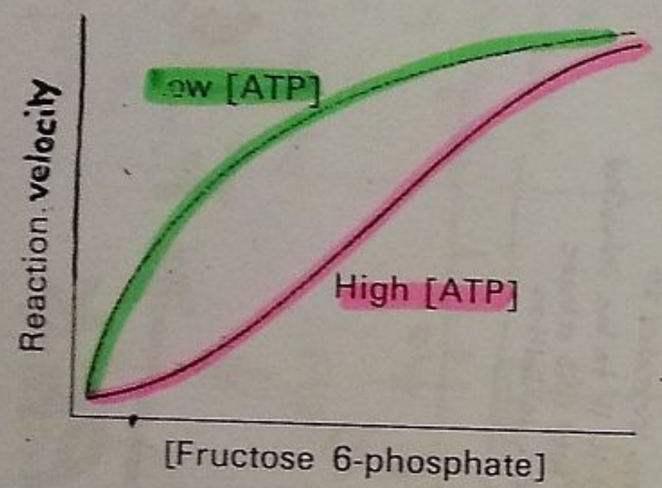


This inc. is enough to overcome the ATP inhibition → enzyme is activated during exercise (a deinhibitor) releases inhibition by ATP.

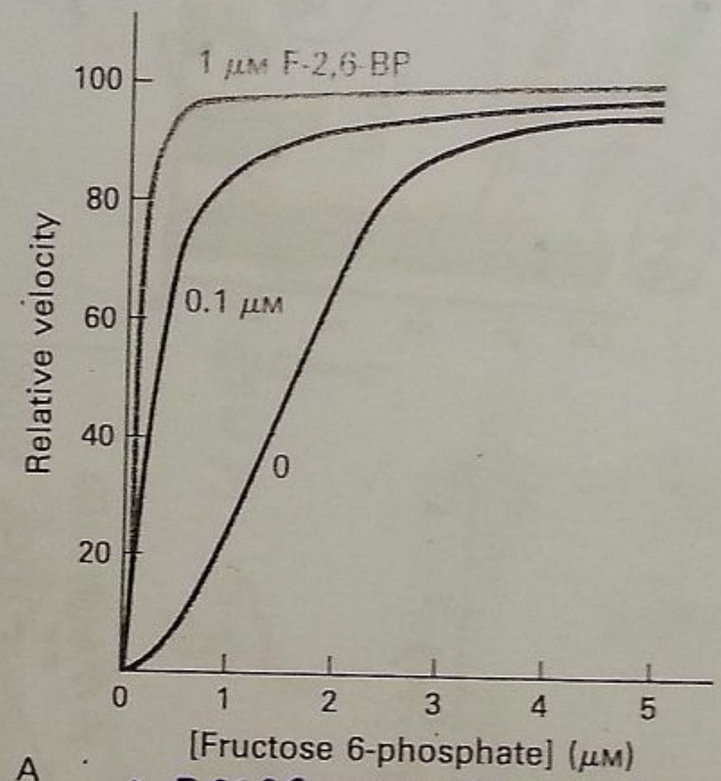
$$F6P + ATP \xrightarrow[PFK]{} F1,6BP + ADP$$

Regulation of PFK

• Fru 2,6 BP:
* There is hormonal regulation

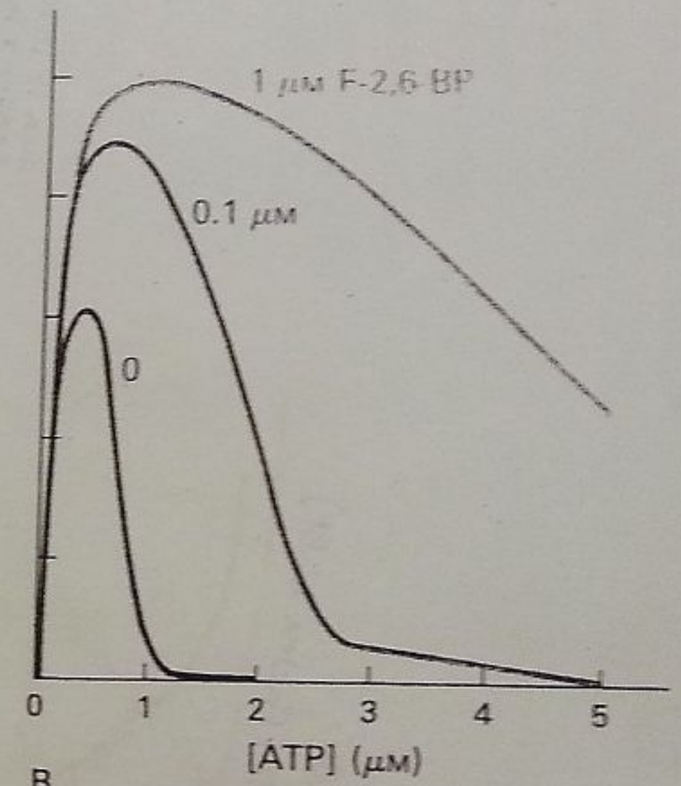


* As ATP conc.↑ → indicates a high rate of glycolysis ∴ it acts as an inhibitor ⇒ formation of fru 1,6 bisphosphate ↓ → reverse rxn is favored



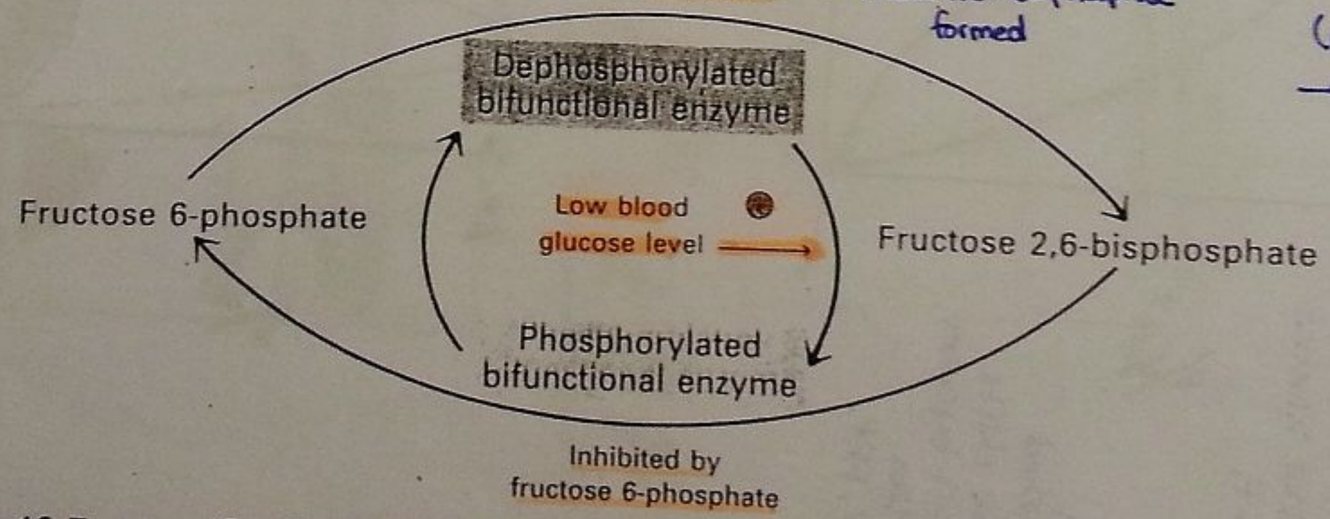
A

* F 2,6 BP activates Pfk1, as its conc ↑ → favors production of fru 1,6 bisphosphate → less fru-6-phosphate formed



B

* as F-2,6 BP conc.↑ → less fru-6-phosphate is formed since it favors forward rxn (production of fru 1,6 bisphosphate) → more ATP is produced in the process



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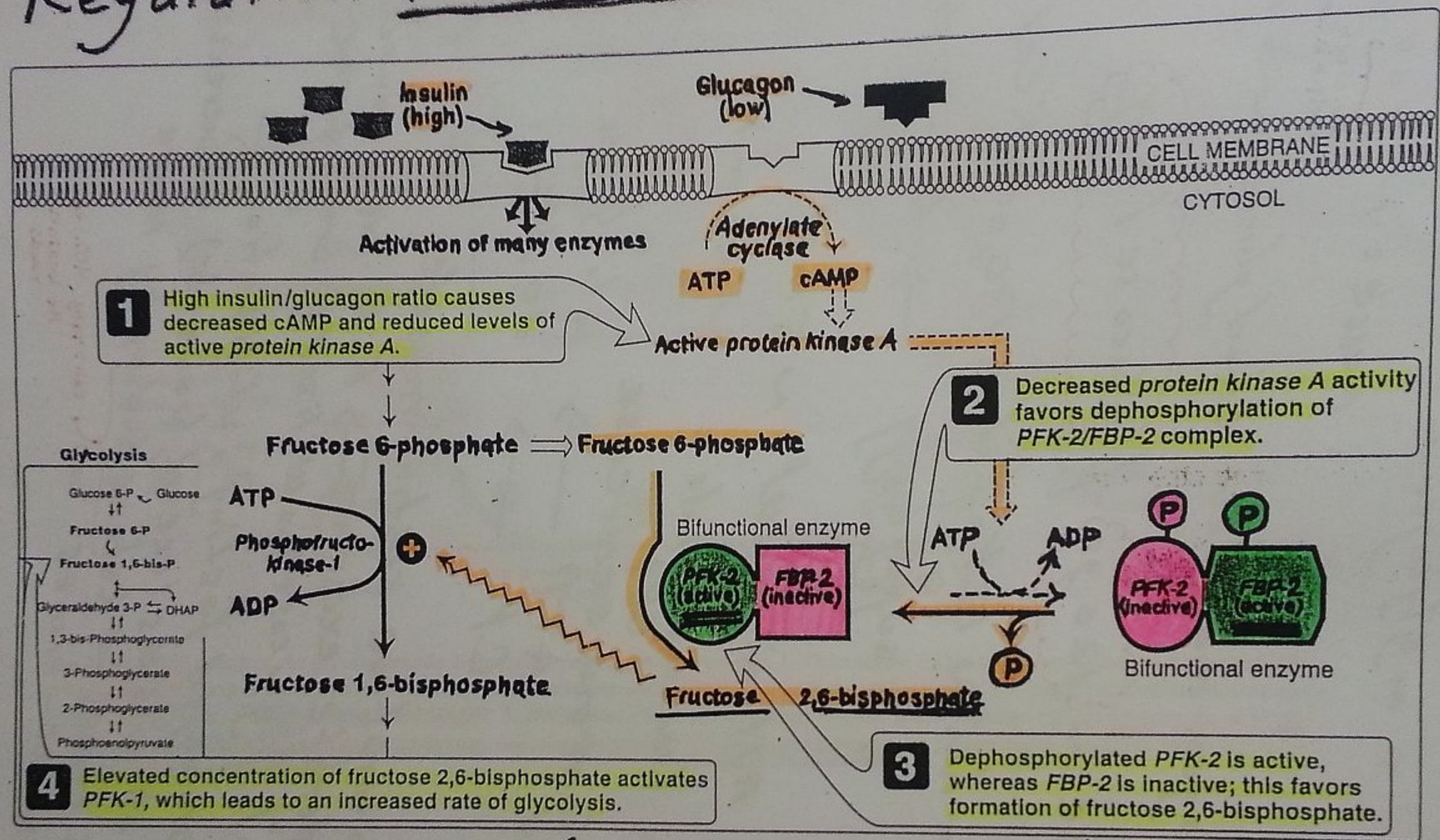
Regulation of Fru-2,6-BP Level



Regulation of PK by phosphorylation – Dephosph

* High insulin → low cAMP → PKA ↓ → Dephosphorylation of PFK-2 / FBP-2 complex → dephosphorylated PFK-2 is active, FBP is inactive

⇒ formation of fructose 2,6 bisphosphate from fructose 6 phosphate

→ Fru 2,6 BP → Activates PFK-1 → glycolysis rate ↑

Distribution of Glucose:

free glucose in E.C.F. = 20 gr ≡ 80 kcal

= " glycogen in liver = ~ 75 gr (10% of net weight in liver)

" " " muscle = ~ 400 gr (2% of net weight in liver)

- liver glycogen maintain blood glucose → 16 h
- Brain use ~ 120 gr of glucose / day
- 70 kg man has ~ 15 Kg fat ≡ 130,000 Kcal
  " supply energy → 60 - 90 days

- Conc of ATP in muscle ~ 5 mM
  " " Creatine phosphate (CP) " ~ 20 mM

Upon vigorous excercise
ATP → 2 to 4 sec
CP → 20 sec

- Post absorptive resting muscle or with moderate excercise → F.A. main source
  80% of glucose is utilized by brain & rbc
- During prolonged fasting, utilization of F.A. by all tissues (except brain + rbc) is increased 4 to 5 times & ketone bodies by more than 100-times (can supply 40% of the brain's needs)

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Glyceraldehyde 3-P dehydrogenase

: converts G3P (aldose) → 1,3BPG (carb with carbon)

⑥

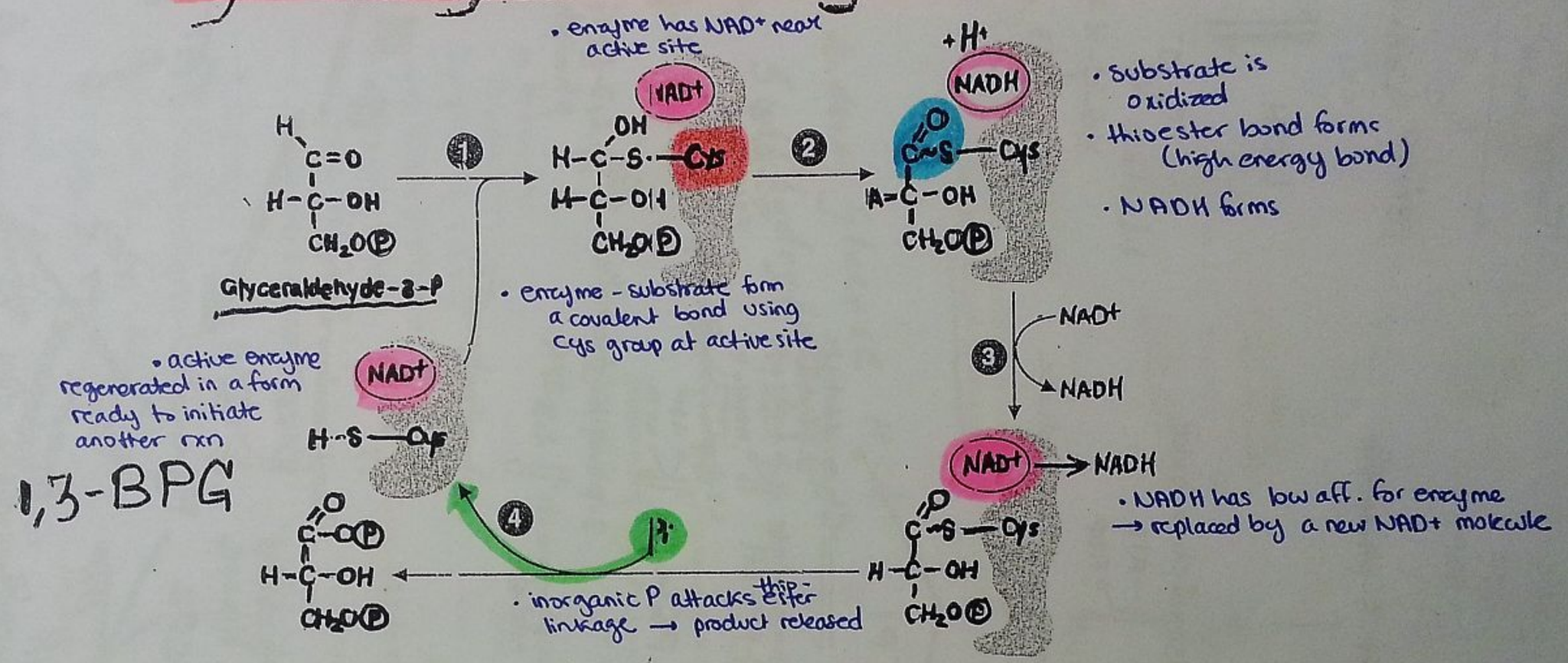
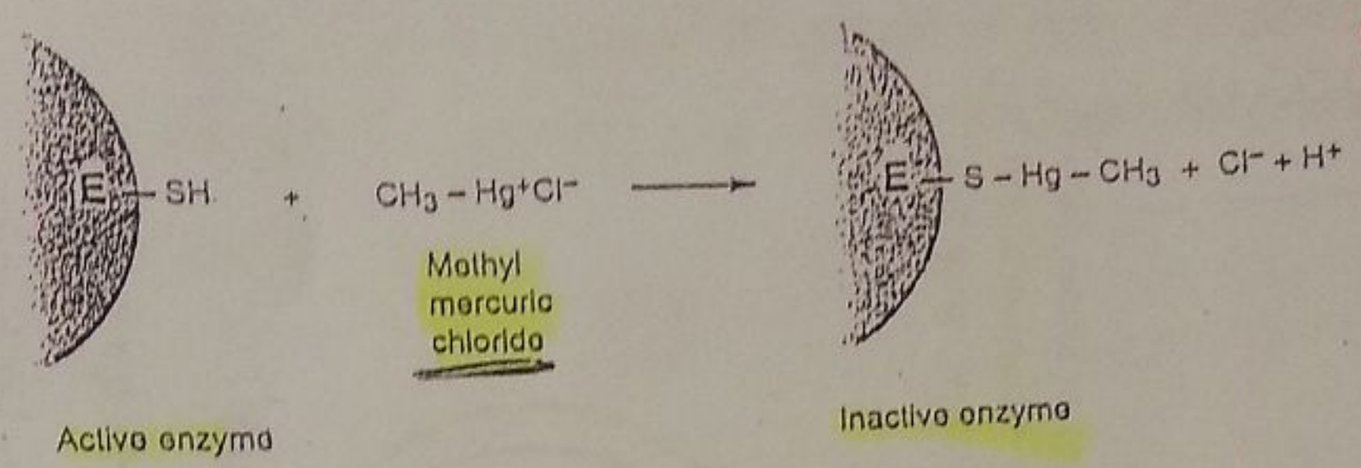


Fig. 22.17. Mechanism of the glyceraldehyde 3-phosphate dehydrogenase reaction. 1. The enzyme forms a covalent linkage with the substrate, using a cysteine group at the active site. The enzyme also contains bound NAD^+ close to the active site. 2. The substrate is oxidized, forming a high-energy thioester linkage (in blue), and NADH. 3. NADH has a low affinity for the enzyme and is replaced by a new molecule of NAD^+. 4. Inorganic phosphate attacks the thioester linkage, releasing the product 1,3 bisphosphoglycerate, and regenerating the active enzyme in a form ready to initiate another reaction.

Glycolysis Can be Inhibited



* mercury in fish, reacts with SH on active enzymes

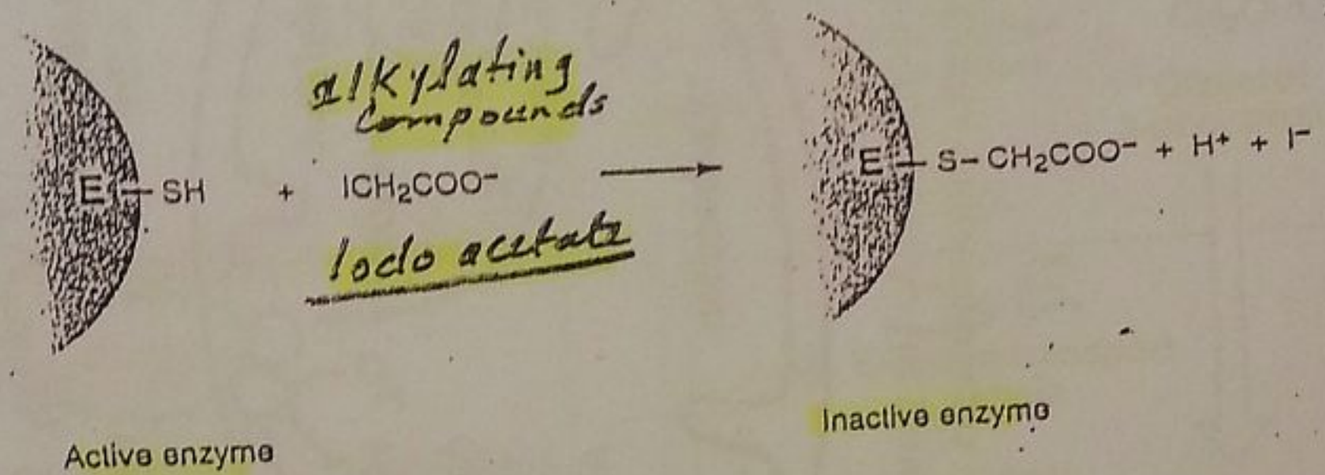


FIGURE 7.11
Mechanism responsible for inactivation of glyceraldehyde-3-phosphate dehydrogenase by sulfhydryl reagents.

1. 2-deoxy Glucose HK↓
2. Sulfhydryl Reagents GADH↓
3. Fluoride Enolase↓

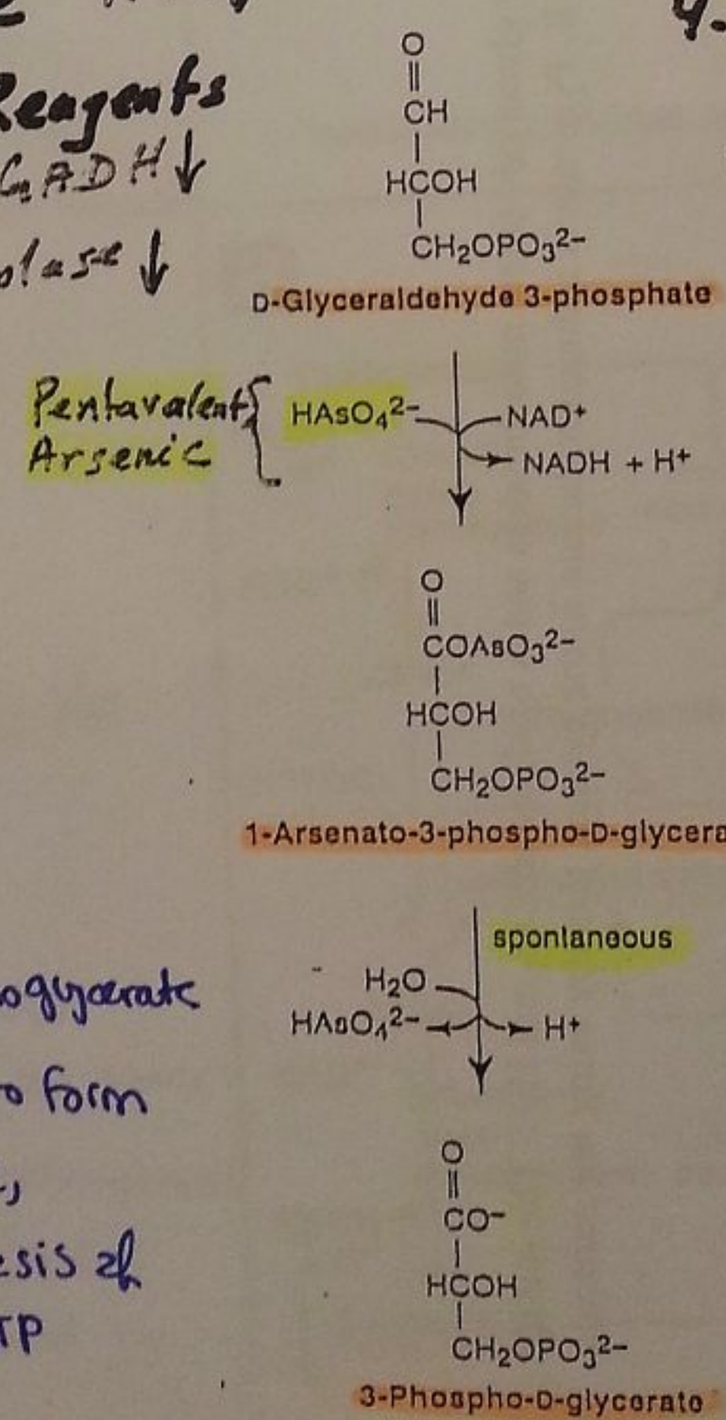


FIGURE 7.12
Arsenate uncouples oxidation from phosphorylation at the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase.

* Pentavalent Arsenic (Arsenate) Can prevent net ATP and NADH production:
- competes with Pi as a substrate for G3P
→ 1Arsenato-3-phosphoglycerate
→ Hydrolyzes readily to form 3Phosphoglycerate, bypassing the synthesis of 1,3 BPG → no ATP formed → cell is deprived of energy.

4. Arsenate Inhibition $HAsO_4^{2-}$

Arsenolysis also interfer with ATP-formation by Ox. phos.
- Can also replace phosphate in ATP
- replaces PO_4 (2 ATPs lost)

Arsenite (trivalent) AsO_2^-

bind both -SH of the cofactor lipoic acid and inhibiting e.g PD, KG-D -- etc.
more TOXIC

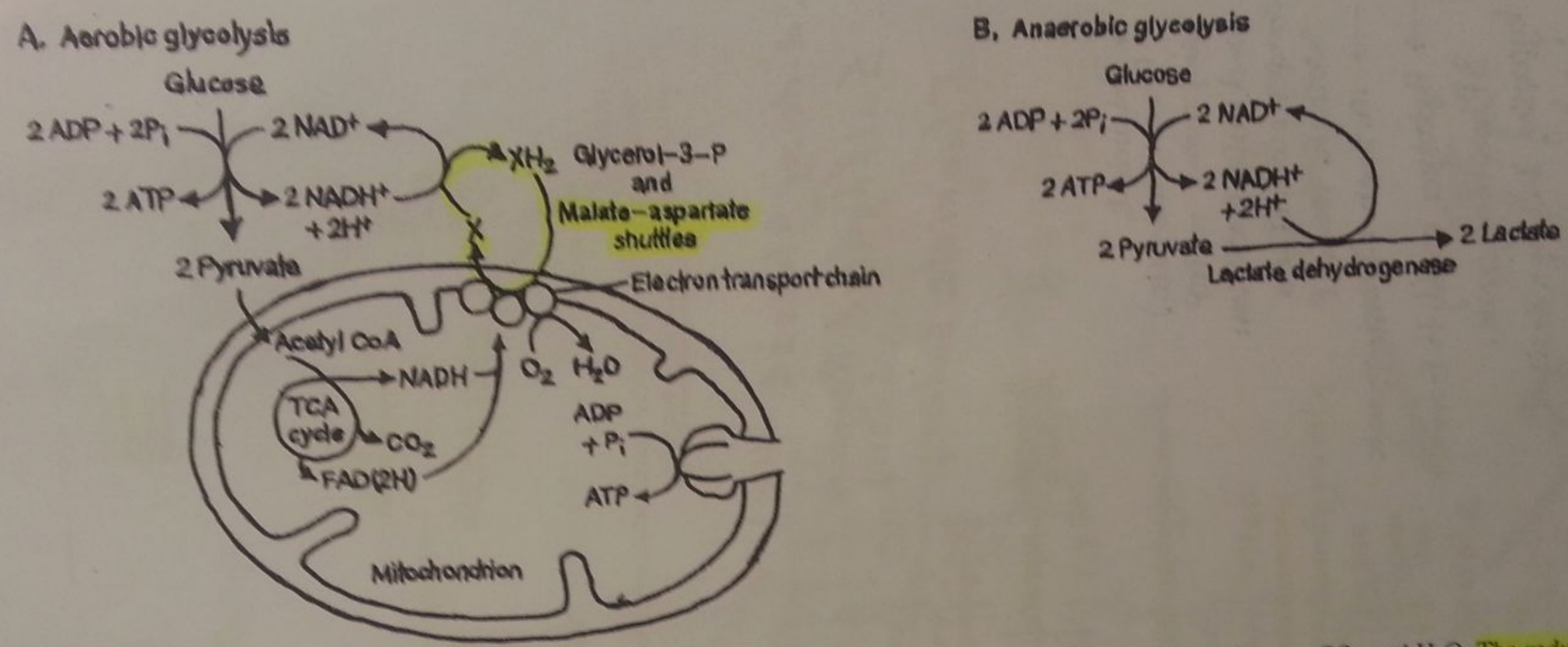
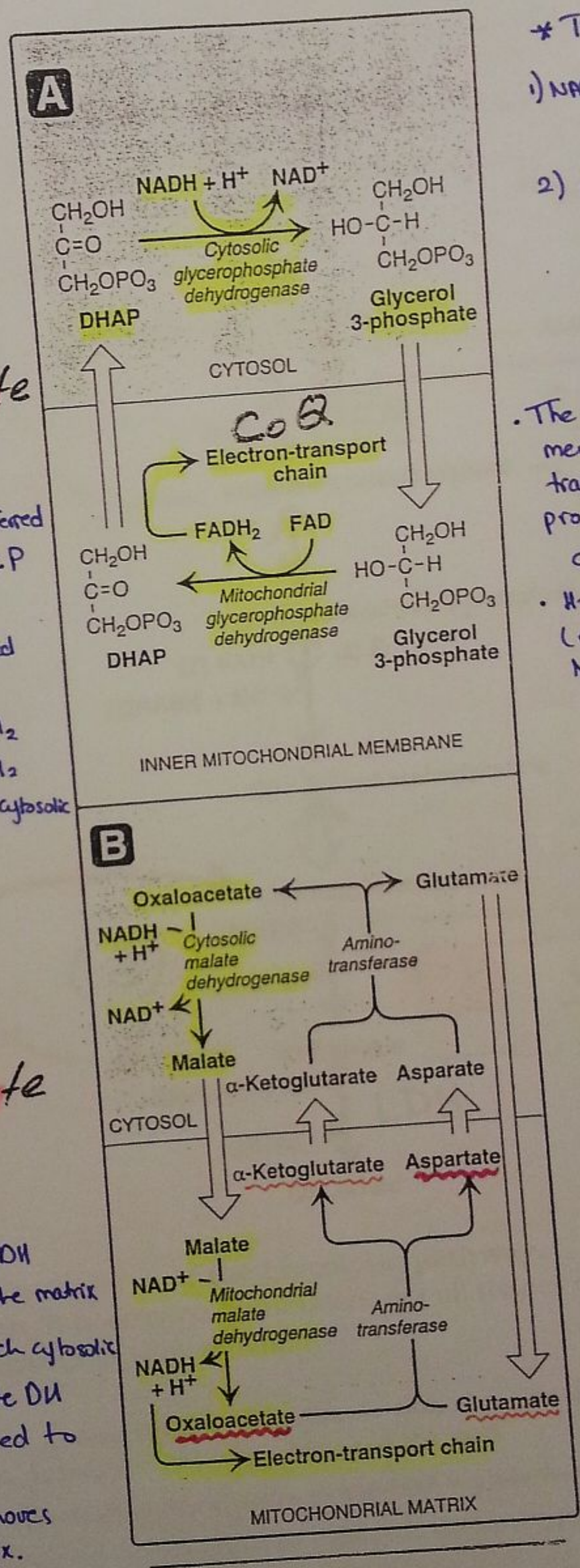


Fig. 22.6. Alternate fates of pyruvate. A. The pyruvate produced by glycolysis enters mitochondria and is oxidized to CO_2 and H_2O. The reducing equivalents in NADH enter mitochondria via a shuttle system. B. Pyruvate is reduced to lactate in the cytosol, thereby using the reducing equivalents in NADH.

Shuttle Pathways for the Transport of Electrons across the Inner mitochondrial membrane :-

* Two major mechanisms of NADH oxidation
1) NADH-linked conversion of pyruvate → lactate
2) Oxidation of NADH via ETC
⟹ This requires the malate-aspartate and glycerol 3 phosphate Substrate Shuttles.



* Glycero-phosphate shuttle

- two electrons are transfered from NADH to dihydroxyacetone P by cytosolic GPDH.
- Glycerol 3 P produced is oxidized by mitochondrial isozyme as FAD is reduced to $FADH_2$
- CoQ of ETC oxidizes $FADH_2$
- two ATPs result from each cytosolic NADH oxidized.

- The inner mitochondrial membrane lacks an NADH transporter ∴ NADH produced in cytosol cannot directly enter the matrix.
- However, two electrons (reducing equivalents) of NADH are transported using shuttles.

* Malate-Aspartate shuttle

- This shuttle produces NADH instead of $FADH_2$ in the matrix
- ∴ it yields 3 ATPs for each cytosolic NADH oxidized by malate DH as oxaloacetate is reduced to malate
- a transport protein moves malate into matrix.