CARBOHYDRATE METABOLISM

Warren Jelinek

I. THE HANDOUT

This handout has five parts:

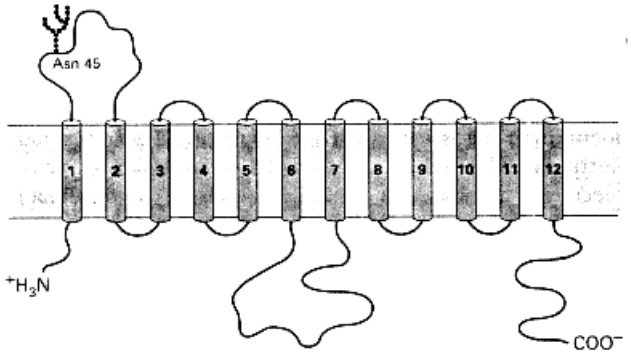
1. a summary of small molecules commonly used in metabolism for the production and utilization of energy by cells (METABOLIC ENERGY),
2. a short synopsis of the metabolic pathways and hormonal regulation of glucose metabolism (SYNOPSIS OF GLUCOSE METABOLISM),
3. a chart showing the five metabolic pathways covered in these lectures (THE PATHWAYS), with each numbered according to the order of presentation (glycolysis # 1 - 11, tricarboxylic acid cycle #12 - 20, pentose phosphate shunt #21 - 28, gluconeogenesis # 29, 30, 9, 8, 7, 6, 5, 4, 31, 32, glycogen metabolism #33 - 39 - Important features of each reaction are described.),
4. a review of the regulated steps in glucose metabolism (IRREVERSIBLE AND REGULATED STEPS OF GLUCOSE METABOLISM), which is intended to assist you in your study,
5. copies of slides shown in class that are not included in your textbook.

II. STUDY QUESTIONS are available on the course web site.

NOTE: You are **NOT** required to learn the chemical structures shown in the lectures. They are included at the end of this handout merely as a reference to aid in your study, if you so desire.

SYNOPSIS OF GLUCOSE METABOLISM

A. Glucose is a chemical fuel which can be used to obtain energy, reducing power and carbon skeletons.
    1. Glucose is the major form in which carbohydrates absorbed through the intestinal epithelium are presented to cells.
        - Common dietary disaccharides from which glucose is derived are:
          sucrose, a disaccharide of glucose and fructose,
          maltose, a disaccharide of glucose,
          lactose, a disaccharide of galactose and glucose.
          The intestinal epithelium splits disaccharides into monosaccharides and transports them to the blood via the portal system.
    2. Different tissues take up glucose from blood at different concentrations depending on their glucose transporters (GLUTs)
        - Blood glucose is maintained at about 5mM.
        - Glucose transporters (GLUT) are integral membrane proteins that have 12 membrane-spanning domains.



Model of a mammalian glucose transporter. The hydrophobicity profile of the protein points to the presence of 12 transmembrane alpha helices. Chemical labeling and tryptic cleavage studies support this postulated topography. [From M. Muekler, C. Caruso S.A. Caldwin, M. Panico, M. Blench, H.R. Morris W.J. Allard G.E. Lienhard and H.F. Lodish, *Science* 229 (1985):941.]

        - GLUT1 and GLUT3 - present on many cells, K_m = 1 mM. Since blood glucose is usually maintained at about 5mM, GLUT1 and GLUT3 transport glucose at a constant rate independent of blood glucose concentration.
        - GLUT 2 - present on liver and pancreatic beta cells, K_m = 15 - 20 mM The rate of entry of glucose into liver and pancreatic beta cells is proportional to the blood glucose levels.
        - GLUT4 - present on muscle and fat cells, K_m = 5 mM
          Insulin causes an increase in the number of GLUT4 molecules in the membranes of muscle and fat cells and thus controls entry of glucose in these cells.
        - GLUT5 - actually a fructose transporter.
    3. Tissue-specific use of glucose as a major fuel
        - Brain and muscle use glucose as a major fuel.
          Brain also uses ketone bodies as fuel during severe starvation
        - Liver does not use glucose as its major fuel. Rather, liver exports glucose for use by other tissues.

B. Glycolysis, the TCA cycle and the pentose phosphate shunt transduce the chemical energy of glucose into two forms:
    1. the high-energy phosphate bond of ATP
    2. the electron donors NADH, $FADH_2$ and NADPH (referred to as reducing equivalents).

C. Glycolysis
    - all cells do glycolysis
    - $C_6 \rightarrow 2\ C_3$
    - aerobic glycolysis produces a net gain of 2 ATP and 2 ($NADH + H^+$)
    - anaerobic glycolysis produces a net gain of 2 ATP (e.g., in red blood cells and exercising muscle) Under anaerobic conditions NADH is used to reduce pyruvate to lactate to regenerate NAD^+ so that glycolysis can continue.

D. Loss of CO_2 and gain of NADH in the conversion of pyruvate to acetyl CoA

E. TCA cycle
    - only to a limited extent in fat cells, which use acetyl CoA primarily to synthesize fat, NOT in red blood cells, which don't have mitochondria
    - limiting in exercising muscle in which the TCA cycle and oxidative phosphorylation can't keep up with the rate at which pyruvate is produced by glycolysis
    - 2 CO_2 released during the final oxidation steps of entering acetate derived from acetyl CoA
    - 1 ATP gained (as GTP)
    - 4 reducing equivalents gained as 3 NADH and 1 $FADH_2$
    - To yield energy indirectly, electrons from NADH and $FADH_2$ are supplied to the mitochondrial electron transport chain (to be discussed in later lectures), which produces ATP at the expense of the ultimate electron acceptor, O_2. MUCH more ATP is obtained from these subsequent electron transport reactions than is directly obtained via glycolysis, the TCA cycle or the pentose phosphate shunt.

F. Pentose Phosphate Shunt
    - happens in all cells, to different extents
    - the oxidative branch yields 2 reducing equivalents, as 2 NADPH, and one molecule of pentose ($C_{5)}$ phosphate from each glucose-6-phosphate
    - NADPH is important for reductive biosynthesis, particularly in fat cells, which produce and consume large amounts in the synthesis of fatty acids. Red blood cells use NADPH to maintain a reduced environment.
    - the non-oxidative branch interconverts phosphate sugars
      $C_5 \leftrightarrows C_3, C_4, C_6, C_7$

G. Glucose is stored as glycogen, a glucose polymer.
    - in muscle, heart, liver, to a limited extent in fat cells - NOT in brain, NOT in red blood cells

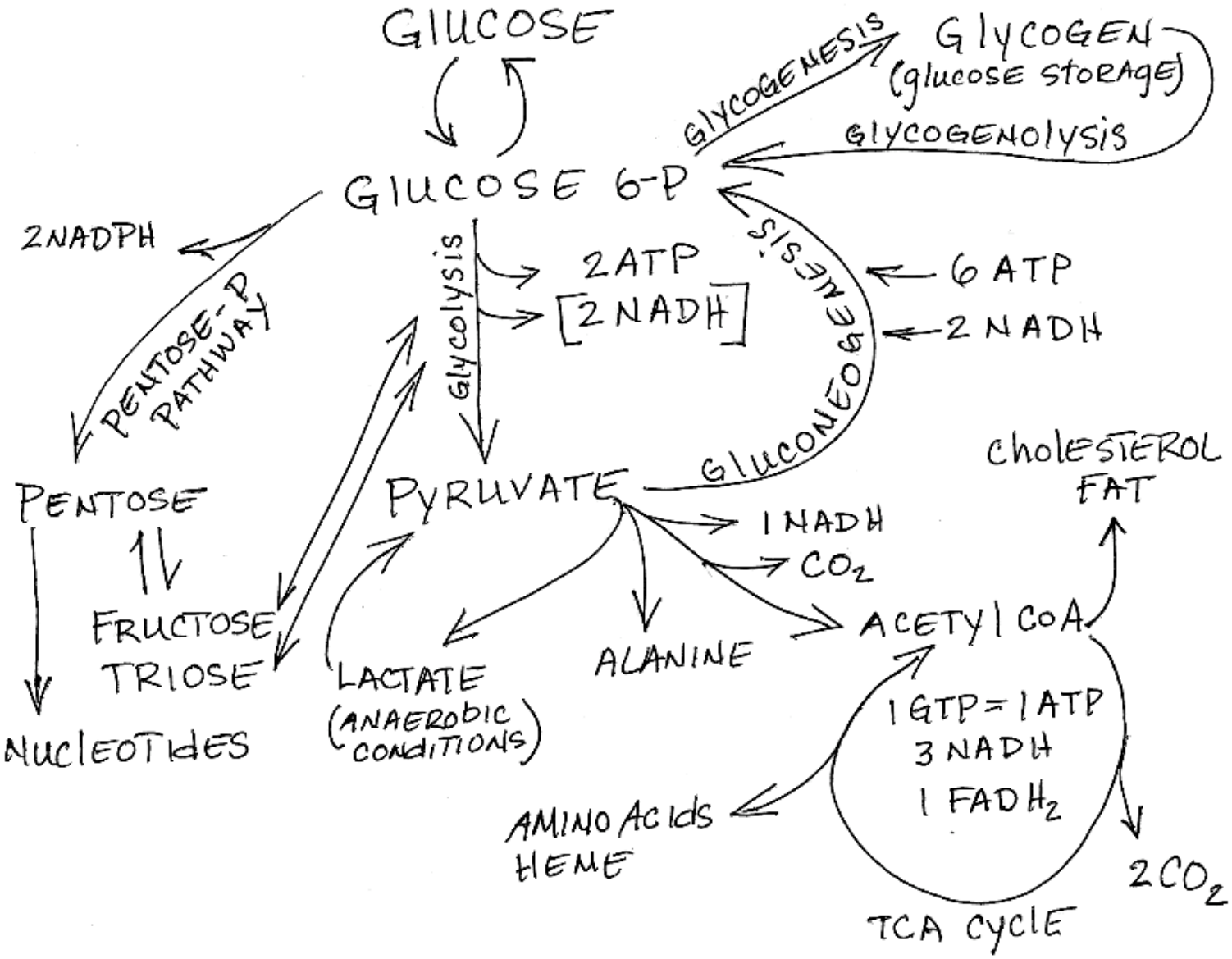
H. Intracellular generation of glucose
    1. Gluconeogenesis
        - in liver, kidney
        - requires a net utilization of energy
        - $C_3 \rightarrow C_6$

2. Glycogenolysis - glycogen breakdown to glucose-1-phosphate and then conversion to glucose-6-phosphate
    - in muscle, heart, liver, to a limited extent in fat cells, NOT in brain, NOT in red blood cells

I. **Only the liver and kidney can generate glucose for export to the blood**.

J. Glucose provides cellular building material as well as fuel. Its breakdown provides precursors for biosynthesis of amino acids, heme, fatty acids, cholesterol and other essential molecules.

K. The centrality of glucose in metabolism requires good homeostatic control of blood glucose at about 5 mM to ensure adequate concentrations in all tissues, especially in the brain and muscles.
    - Coordination of glucose breakdown (glycolysis), glucose synthesis (gluconeogenesis) and glucose storage and release (glycogen metabolism) is achieved by hormonal (insulin, glucagon, epinephrine and norepinephrine) regulation (resulting in the covalent modification of essential enzymes -primarily phosphorylation) and by tissue-specific differences in the distribution of key enzymes.
    - Pancreatic alpha cells secrete glucagon in response to low blood glucose and glucagon causes the liver to release glucose to the blood.
    - Pancreatic beta cells secrete insulin in response to high blood glucose. Insulin promotes uptake of glucose by muscle and fat cells and promotes glucose storage in liver.
    - The pancreatic insulin-secreting beta cells and the glucagon-secreting alpha cells are in a co-regulatory circuit. Secretion of insulin and associated molecules by the beta cells inhibits the secretion of glucagon from the alpha cells. Thus, increased insulin release is coupled to reduced glucagon release (high blood glucose). Recent evidence suggests that zinc, secreated with insulin from the beta cells may be the inhibitor of glucagon secretion from the alpha cells. Glucagon, secreted by the alpha cells (low blood glucose) causes the beta cells to secrete a low level of insulin. This relatively low level of insulin secretion is not sufficient to counteract the higher levels of glucagon secreted in response to low blood glucose. Various speculations exist concerning the physiological significance of the low insulin secretion in response to glucagon.


GLUCOSE
GLYCOGENESIS
GLYCOGEN
(glucose STORAGE)
GLYCOGENOLYSIS
GLUCOSE 6-P
2NADPH
PENTOSE-P PATHWAY
Glycolysis
2ATP
[2 NADH]
GLUCONEOGENESIS
6 ATP
2 NADH
PENTOSE
PYRUVATE
CHOLESTEROL
FAT
1 NADH
CO_2
FRUCTOSE
TRIOSE
ACETYL CoA
LACTATE
(ANAEROBIC CONDITIONS)
ALANINE
NUCLEOTIDES
1 GTP = 1 ATP
3 NADH
1 $FADH_2$
AMINO ACIDS
HEME
$2CO_2$
TCA CYCLE

GLUCOSE METABOLISM IN VARIOUS TISSUES*							
	Glycolysis	TCA Cycle	Gluconeogenesis	Glycogenesis	Glycogenolysis	Pentose Shunt	Comment
Liver	yes	yes	yes	yes	yes	yes very active	stores glucose as glycogen and recovers it for release to blood as needed; synthesizes glucose from 3-carbon precursors and releases it to blood as needed; uses glucose breakdown products for syntheses of other biological molecules, e.g., fats, steroids, amino acids, heme
Brain	yes	yes	no	no	no	yes	highly dependent on glucose which it neither stores nor synthesizes; can also use ketone bodies as fuel during starvation
Muscle & Heart	yes	yes	no	yes	yes	yes	stores glucose as glycogen and recovers glucose from glycogen for use on site, i.e., does not release glucose into blood; during vigorous muscle activity glucose utilization is essentially anaerobic because the TCA cycle can't keep pace with the high rate of glycolysis and lactate is produced to regenerate NAD
Red Blood Cells	yes	no	no	no	no	yes	no mitochondria, therefore, anaerobic use of glucose; does not store glucose; the pentose phosphate shunt is particularly important to generate reducing power which keeps proteins in the reduced state and heme iron in the ferrous state in this highly oxidizing environment; converts 1,3-bisphosphoglycerate to 2,3-bisphosphoglycerate for use by hemoglobin
Adipose	yes	limited	no	limited	limited	yes very active	glycolytic acetyl CoA is used primarily to synthesize fat

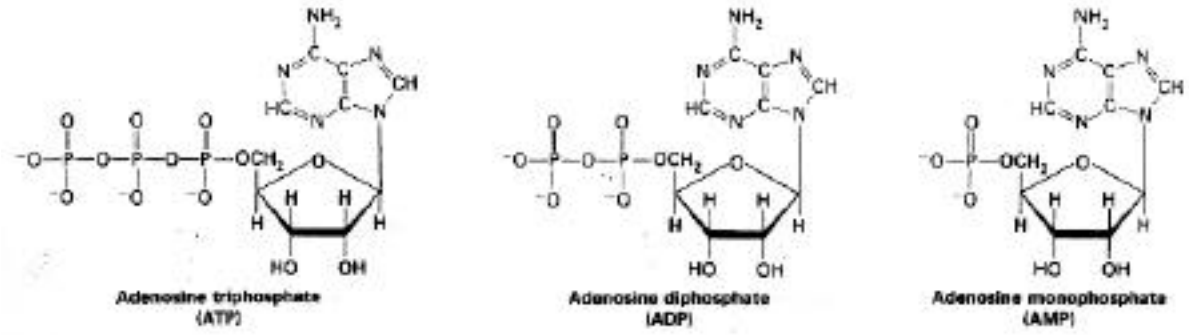
* These pathways are regulated to meet the physiological demands of both the tissue and the entire body. For example, in the well fed state the liver is glycolytic (breakes down glucose), glycogenic (synthesizes glycogen), and has an active TCA cycle; in the fasting state it is gluconeogenic (synthesizes glucose), glycogenolytic (breaks down glycogen), and has a relatively inactive TCA cycle.

METABOLIC ENERGY

A. All living organisms require a continual input of energy to maintain the multitude of metabolic reactions in a state far from equilibrium (equilibrium = death).

Three major purposes for energy in biological organisms:
- Mechanical Work - e.g., muscle contraction
- Active Transport - to maintain chemical balance, e.g., cells need to continually pump out Na^+ ions which are taken in as part of transport mechanisms for other molecules - achieved by the Na^+/K^+ ATPase, which uses energy to pump Na^+ against a concentration gradient. Active transport accounts for about 10% - 30% of the energy needs of an organism.
- Synthesis of essential biological molecules.

B. Adenosine Triphosphate [ATP] is the universal currency of energy in biological processes within cells. It is the link between energy-producing and energy-utilizing systems. Its hydrolysis yields useful energy.



$$ATP + H_2O \rightarrow ADP + P_i + H^+ + \text{energy (7.3 kcal/mol)}$$

C. Structural basis for the phosphoryl transfer potential of ATP
- electrostatic repulsion between proximal negative charges
- ADP and P_i have a larger number of resonance forms than ATP

D. The energy liberated in the hydrolysis of ATP can be harnessed to drive reactions that require an energy input.
- Reactions that wouldn't proceed spontaneously, because they required an input of energy, can proceed if they are coupled to ATP hydrolysis.
- ATP hydrolysis shifts the equilibria of coupled reactions. The equilibrium of a reaction is changed by 10^8 per ATP molecule hydrolyzed. Thus, if 3 molecules of ATP are hydrolysed in a coupled reaction, the equilibrium of the reaction would be changed by 10^{24}.

E. ATP is formed from ADP and P_i when fuel molecules are oxidized by chemotrophs (or when light is trapped by phototrophs)

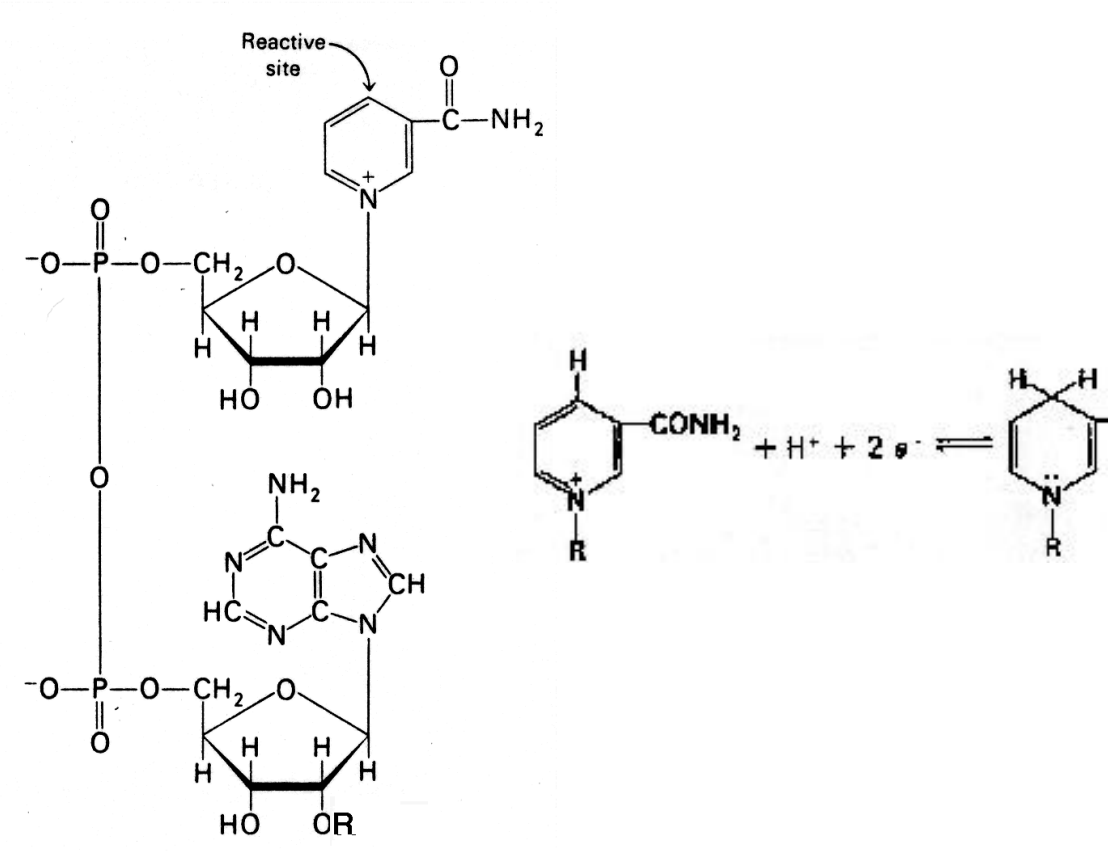
This ATP-ADP cycle is the fundamental mode of energy exchange in biological systems.

- ATP turnover is high. The half-life of an ATP molecule in many tissues is approximately one minute.
- A resting human uses approximately 40 kg of ATP in 24 hours = approximately 1000 tons in a lifetime. During strenuous exertion it can be as high as 0.5 kg / min.

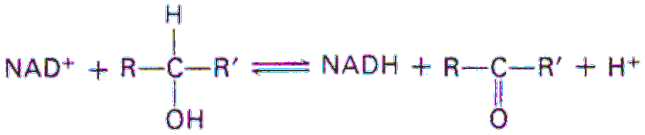
F. NADH and $FADH_2$ are the major electron carriers in the oxidation (dehydrogenation) of fuel molecules.

In aerobic organisms the ultimate electron acceptor is O_2. However, electrons are not transferred directly from fuel molecules and their breakdown products to O_2. They are first transferred to the electron carriers (substrate-level oxidations). The reduced forms of these carriers then transfer their high-potential electrons to O_2 by means of an electron transport chain located in the inner membrane of the mitochondria.

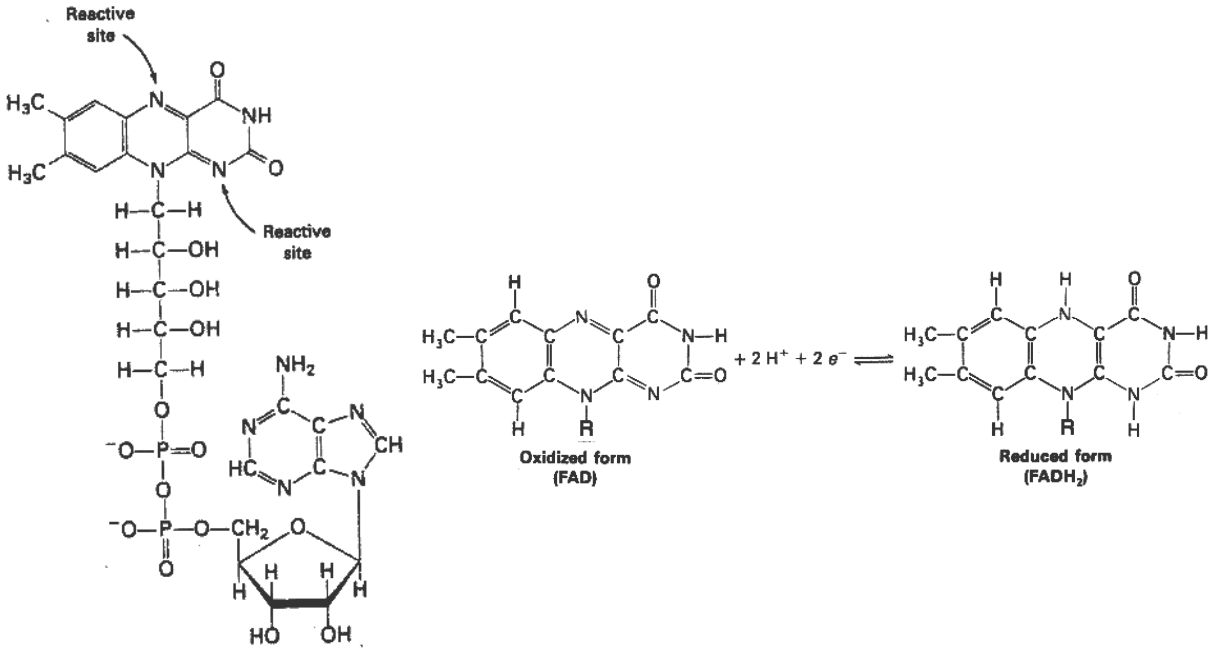
1. Nicotinamide adenine dinucleotide [NAD^+] structure - in NAD^+ R = H, in $NADP^+$ R = PO_3^{2-}
   - In the oxidation (dehydrogenation) of a substrate, the nicotinamide ring of NAD^+ accepts a hydrogen ion and two electrons - the equivalent of a hydride ion.
   - The reduced form of this electron carrier is called NADH. In the oxidized form, the pyridine ring nitrogen is tetravalent and carries a positive charge. In the reduced form, NADH, the nitrogen atom is trivalent.



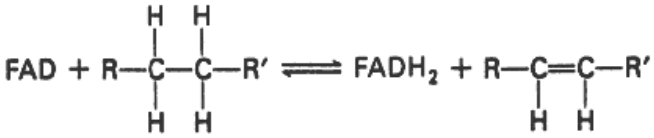
- Reaction type that uses NAD^+ (typically, but not exclusively, oxidation of an alcohol to a carbonyl).



2. Flavin adenine dinucleotide [FAD]
   - The reactive part of FAD is its isoalloxazine ring. It accepts two electrons, and two protons.

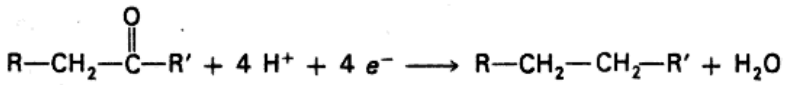


   - Reaction type that uses FAD (generally, but not exclusively, a dehydrogenation of a saturated compound with the formation of a double bond)



G. NADPH is the major electron donor in reductive biosynthesis.
   - In most biosynthetic reactions the precursors are more oxidized than the products so **reducing power** is required

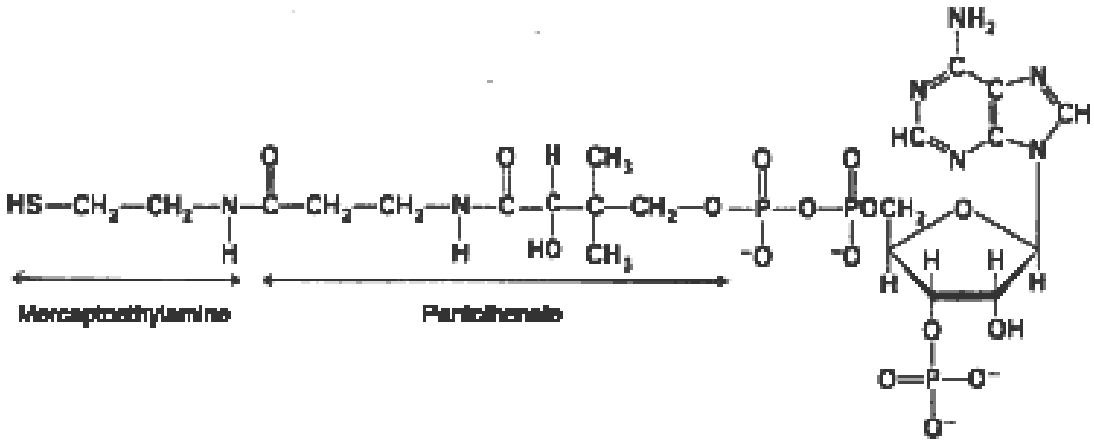
- e.g., in fatty acid biosynthesis a keto group is sequentially reduced via an alcohol and an unsaturated aliphatic chain.



- NADPH differs from NADH in that the 2'-OH of its adenosine moiety is esterified with phosphate. This extra phosphate is a tag that directs this reducing agent to discerning biosynthetic enzymes.
- NADPH carries electrons in the same way as NADH.
- **NADPH is used almost exclusively for reductive biosynthesis, whereas NADH is used primarily for the generation of ATP.**

H. Why have both $NADP^+$ and NAD^+ ?
- The cellular ratio of $NADP^+/NADPH = 0.014$
- The cellular ratio of $NAD^+/NADH = 700$
- The cell can thus maintain a high level of reducing equivalents for biosynthetic reactions while simultaneously maintaining high capacity for substrate-level oxidations so that energy can be generated.

I. Coenzyme A (A for *acetylation*) is a universal carrier and donor of acyl groups,



- The terminal sulfhydryl group is the reactive site, which forms thioesters with acyl (not only acetyl) groups.

J. Acetyl CoA is a common intermediate in different catabolic pathways.
- glycolysis, beta-oxidation of fatty acids and amino acid degradation all supply the cellular pool of acetyl CoA.

THE PATHWAYS

I. **The major intracellular form of glucose is glucose-6-phosphate**

1. Phosphorylation of glucose to form glucose-6-phosphate by **hexokinase**:
   - irreversible, regulated step
   - ATP is expended
   - feedback inhibited by glucose-6-phosphate, the product of the reaction
   - relatively low K_m for glucose (about 0.1 mM)
   - Glucose-6-phosphate, unlike glucose, is negatively charged, which prevents its exit from cells.
   - ***The liver is a glucose buffer for the blood. Its activity assures constant glucose concentration (approx. 5mM) in the blood.***
     - **Glucokinase,** a liver-specific enzyme whose biosynthesis is induced by insulin in response to high blood glucose levels, has a high K_m (about 10 mM) and high V_{max} for glucose, and is not product-inhibited. The liver does not use glucose as its preferred fuel. Rather, it stores glucose as glycogen when blood glucose levels are high (e.g., after a meal), and recovers glucose from the stored glycogen and releases it into the blood when blood glucose is low (e.g., during fasting). The high K_m and inducibility of glucokinase allows the liver to store glucose as glycogen only when blood glucose levels are high, conserving glucose for other tissues that use it as a primary fuel (e.g., brain, heart, white muscle) when blood glucose levels are low. The non-product-inhibition of glucokinase allows the liver to capture glucose for storage as glycogen even when its intracellular levels of glucose-6-phosphate are high. Glucokinase gene transcription is repressed by glucagon, an hormone secreted by the pancreatic alpha cells in response to low blood glucose levels. When glucose levels are limited (low blood glucose) existing Glucokinase is bound by the Glucokinase Regulatory Protein [GKRP], which inactivates and sequesters it in the cell nucleus, thereby preventing the glucose produced by gluconeogenesis and glycogenolysis from being rephosphorylated and used by the liver. Glucokinase gene transcription is stimulated by insulin, an hormone secreted by the pancreatic beta cells in response to high blood glucose levels.
     - The pancreatic insulin-secreting beta cells and the glucagon-secreting alpha cells are in a co-regulatory circuit. Secretion of insulin and associated molecules by the beta cells inhibits the secretion of glucagon from the alpha cells. Thus, increased insulin release is coupled to reduced glucagon release (high blood glucose). Recent evidence suggests that zinc, secreated with insulin from the beta cells may be the inhibitor of glucagon secretion from the alpha cells. Glucagon, secreted by the alpha cells (low blood glucose) causes the beta cells to secrete a low level of insulin. This relatively low level of insulin secretion is not sufficient to counteract the higher levels of glucagon secreted in response to low blood glucose. Various speculations exist concerning the physiological significance of the low insulin secretion in response to glucagon.
     - The major function of liver glycolysis is to obtain carbon skeletons and energy for the synthesis of biomolecules that the liver exports for use or storage (e.g., fat) by other tissues.

II. **Glycolysis occurs in the cytosol, yielding 2 ATP, 2 pyruvate and 2 (NADH + H^+) from each glucose molecule**

The first steps of glycolysis convert glucose-6-phosphate into a form that can be readily cleaved into phosphorylated three-carbon units. High energy phosphate (as ATP) is then generated from the three-carbon units.

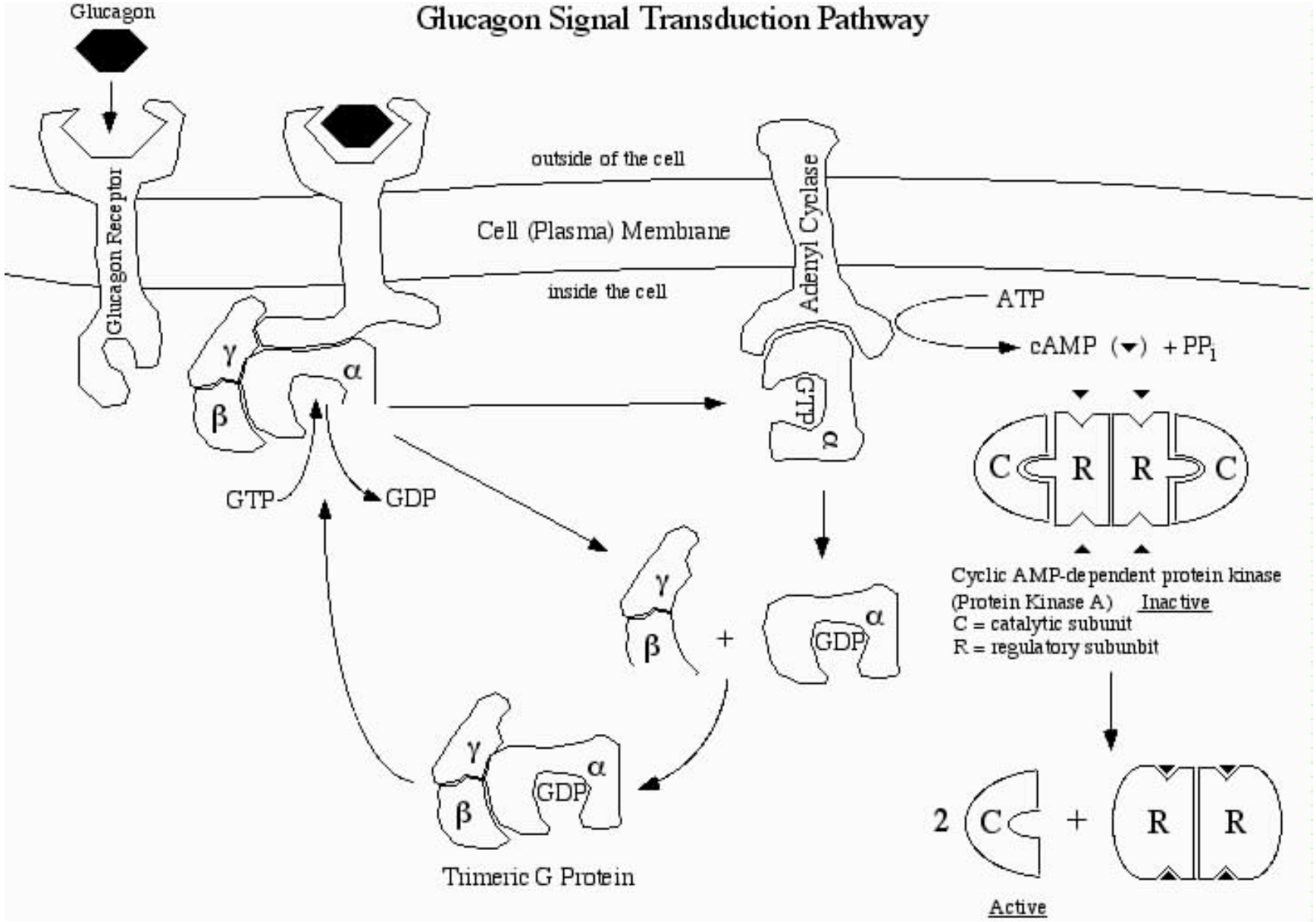
2. Isomerization of glucose-6-phosphate (aldose) to fructose-6-phosphate (ketose) by **phosphoglucose isomerase**:
   - freely interconverted

3. Phosphorylation of fructose-6-phosphate to form fructose-1,6-bisphosphate by **phosphofructokinase 1** [PFK1]: ***THIS IS THE RATE-LIMITING STEP OF GLYCOLYSIS AND THE MOST IMPORTANT SITE OF REGULATION*.**
   - irreversible, regulated step
   - ATP is expended
   - responds to cell energy charge (ATP/AMP ratio), so that allosteric inhibition by ATP is antagonized by AMP
   - ATP lowers the affinity (increases the K_m) of the enzyme for its substrate, fructose-6-phosphate, thereby slowing glycolysis when the energy charge of the cell is high.
   - Citrate, an early TCA cycle intermediate, also inhibits PFK1, by increasing its sensitivity to ATP. A high level of citrate indicates that alternate fuels such as fatty acids and ketone bodies (which will be discussed in later lectures) are available.
   - inhibited by low pH, thereby guarding against excessive lactate formation from glycolysis, which would cause a further drop in blood pH (lactic acidosis)
   - activated allosterically by fructose-2,6-bisphosphate which increases the affinity for fructose-6-phosphate (lowers the K_m) and decreases the sensitivity to ATP inhibition

3a. Phosphorylation of fructose-6-phosphate to form fructose-2,6-bisphosphate by **phosphofructose kinase 2** [PFK2], a remarkable protein with TWO, distinct active sites (bifunctional enzyme). One site has phosphofructose kinase (phosphofructose kinase 2 [PFK2]) activity and the other site has fructose bisphosphatase (fructose bisphosphatase 2 [FBPase 2] activity.
   - Fructose-2,6-bisphosphate is the most important regulator of glycolysis in the liver. Its abundance is a reflection of whether blood glucose levels are high or low (see below).
   - "Feed-forward" allosteric activation by fructose-6-phosphate
   - The kinase reaction is favored by the dephosphorylated form of the enzyme.
   - The phosphatase reaction is favored by the phosphorylated form of the enzyme. This is NOT a reversal of the kinase reaction (don't re-create ATP).
   - The liver enzyme is phosphorylated by cAMP-dependent protein kinase.
   - cAMP levels rise in response to hormones such as glucagon and epinephrine and norepinephrine, (receptors for which activate adenylate cyclase), or in response to events that inhibit cAMP phosphodiesterase (e.g., caffine intake). cAMP-dependent protein kinase consists of catalytic subunits and cAMP-binding regulatory subunits. The catalytic subunit is inactive when bound to the regulatory subunit, which dissociates when it binds cAMP.
   - Glucagon, secreted by pancreatic alpha cells in response to low blood glucose, activates cAMP-dependent protein kinase, switching phosphofructokinase 2 to phosphatase mode, and preventing build-up of fructose-2,6-bisphosphate, thus slowing glycolysis and conserving blood glucose at step #3
   - Conversely, when blood glucose is high, glucagon levels are low, insulin levels are high, the enzyme is dephosphorylated, synthesis of fructose-2,6-bisphosphate is favored, and glycolysis is speeded up at step #3.

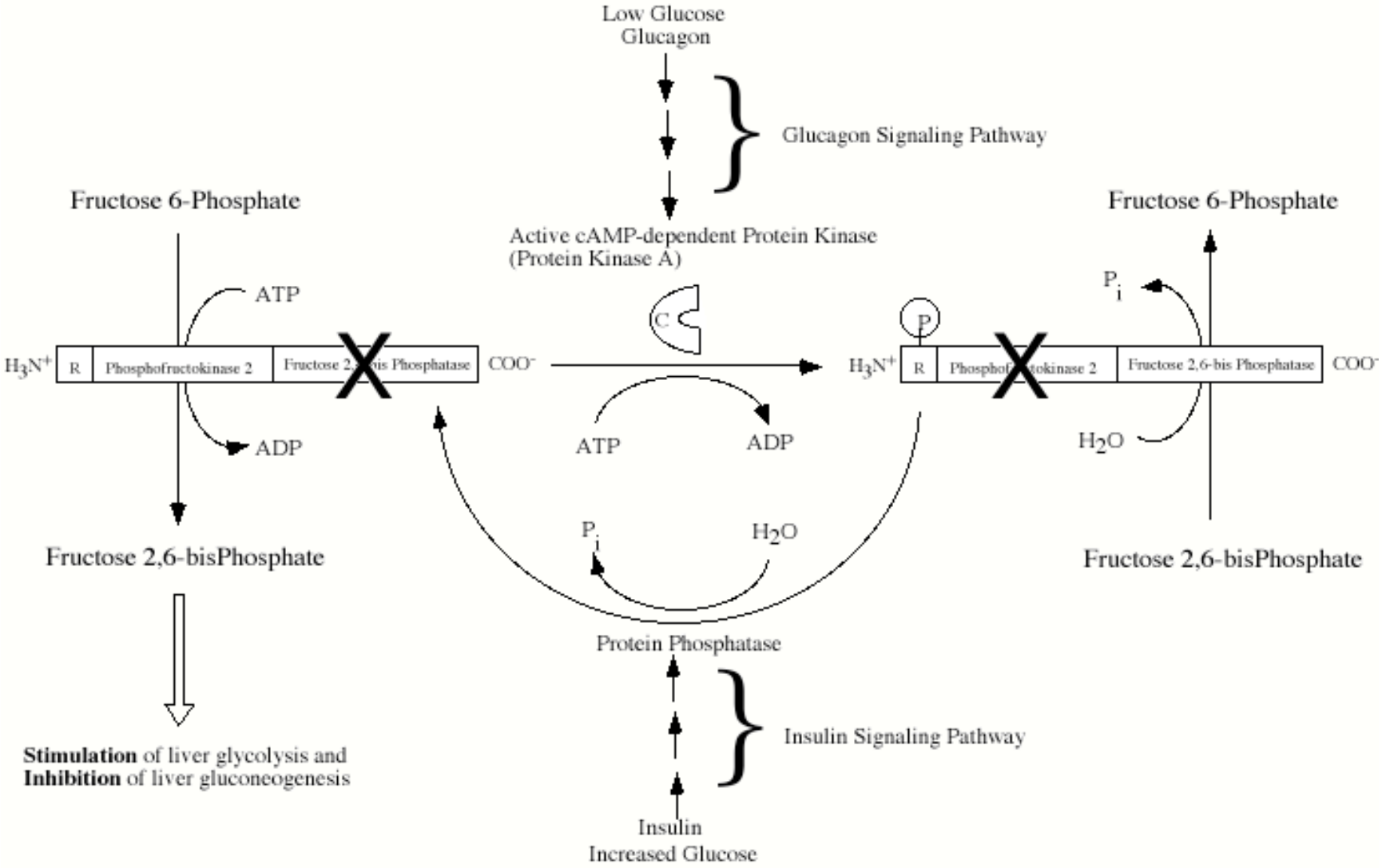
In summary, the PFK2 kinase activity predominates in the "fed" state, the phosphatase activity predominates in the "starved" state.

   - The muscle isoform of PFK2/FBPase (bifunctional enzyme) is not phosphorylated (not regulated) in response to the starved state (muscle does not have glucagon receptors). Furthermore, the skeletal muscle isoform does not have a site for phosphorylation by cyclic AMP-dependent protein kinase. In skeletal muscle, fructose 6-phosphate is the major regulator of PFK2/FBPase. However, the primary regulator of muscle glycolysis is believed to be ATP (the supply of energy).
   - The PFK2 activity of the cardiac muscle isoform of PFK2/FBPase is STIMULATED


Glucagon
Glucagon Signal Transduction Pathway
Glucagon Receptor
outside of the cell
Cell (Plasma) Membrane
inside the cell
Adenyl Cyclase
ATP
cAMP (▾) + PP1
γ
α
β
GTP
GDP
GTP
α
C R R C
Cyclic AMP-dependent protein kinase
(Protein Kinase A) Inactive
C = catalytic subunit
R = regulatory subunbit
γ
β
+
GDP
α
γ
β
GDP
α
Trimeric G Protein
2 C + R R
Active

Regulation of the Bifunctional Enzyme Phosphofructokinase 2 / Fructose 2,6-bis Phosphatase

Phosphorylation of the bifunctional enzyme by cyclic AMP-dependent protein kinase (protein kinase A) lowers the activity of Phosphofructokinase 2 and increases the activity of Fructose 2,6-bis Phosphatase. Dephosphorylation by an insulin-activated protein phosphatase lowers the activity of Fructose 2,6-bis Phosphatase and increases the activity of Phosphofructokinase 2.



Stimulation of liver glycolysis and
Inhibition of liver gluconeogenesis

by phosphorylation by cyclic AMP-dependent protein kinase (it has a different phosphorylation site than the liver isoform), resulting in an increased rate of fructose 2,6-bisphosphate synthesis and stimulation of phosphofructokinase 1 activity. The rate of heart muscle glycolysis therefore increases upon phosphorylation of PFK2/FBPase. Epinephrine causes an increase in heart rate and an increase of glycolysis (by causing an increase in fructose 2,6-bisphosphate concentration to stimulate phosphofructokinase 1) to supply extra energy for the increased heart rate. Skeletal muscle PFK2/FBPase, because it does not have a phosphorylation site for cyclic AMP-dependent protein kinase, is not affected by epinephrine, but liver PFK2 activity is inhibited, thereby slowing liver glycolysis in response to epinephrine.

4. Cleavage of fructose-1,6-bisphosphate to form dihydroxyacetone phosphate and glyceraldehyde-3-phosphate [G3P] by **aldolase**:
   - reversible
   - G3P is on the direct pathway of glycolysis

5. Interconversion of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate by **triose phosphate isomerase**:
   - reversible

6. Oxidation of glyceraldehyde-3-phosphate to form 1,3-bisphosphoglycerate [1,3-BPG], a high-potential phosphorylated compound, by **glyceraldehyde-3-phosphate dehydrogenase**:
   - reversible
   - ATP NOT expended - part of the energy obtained in the oxidation of G3P is harvested as an electron pair donated to NAD^+ - the resulting NADH will be recycled back to NAD^+ at reaction #11 under anaerobic conditions
   - Carbon 1 of 1,3-BFG is at the oxidation level of a carboxylic acid. The phosphoryl group of this mixed anhydride has high transfer potential.
   - 1,3-bisphosphoglycerate is converted to form large amounts of 2,3-bisphosphoglycerate in red blood cells by bisphosphoglycerate mutase. 2,3-bisphosphoglycerate regulates the hemoglobin/O_2 dissociation curve - deficiency of glycolytic enzymes can cause hemolytic anemia. (Red blood cells, whose primary function is to supply oxygen to tissues, are themselves restricted to anaerobic metabolism, since they lack mitochondria.)

7. Transfer of the high-energy phosphate bond from 1,3-bisphosphoglycerate to form ATP and 3-phosphoglycerate by **phosphoglycerate kinase**:
   - reversible
   - substrate-level phosphorylation; ATP is produced

8. Interconversion of 3-phosphoglycerate and 2-phosphoglycerate by **phosphoglycerate mutase**:
   - reversible

9. Dehydration of 2-phosphoglycerate to form phosphoenolpyruvate [PEP] by **enolase**:
   - reversible
   - the dehydration elevates the transfer potential of the phosphoryl group

10. Transfer of high-energy phosphate bond from phosphoenolpyruvate to form ATP and pyruvate by **pyruvate kinase**:
    - essentially irreversible, highly regulated step
    - substrate-level phosphorylation; ATP is produced
    - "feed forward" activation by fructose-1,6-bisphosphate
    - liver isozyme shows cooperative phosphoenolpyruvate binding, allosteric inhibition by ATP and alanine (an amino acid synthesized from pyruvate in one metabolic step)
    - inhibition by glucagon-triggered phosphorylation - This slows glycolysis in the liver when blood glucose is low, thereby conserving glucose for use by tissues that use it as a primary fuel. Recall that PFK2 (step 3) is also phosphorylated, and its kinase

activity is reduced, by the same mechanism, in response to low blood glucose levels.
- muscle isozyme is NOT regulated by phosphorylation
- A rare, hyperactive mutant pyruvate kinase is harmful to red blood cells because it depletes the precursor pool, diverting substrate from the synthesis of 2,3-bisphosphoglycerate, thereby impairing hemoglobin function.

11. Regeneration of NAD^+ through reduction of pyruvate (the electron acceptor) to form lactate by **lactate dehydrogenase** (fermentation):
    - reversible
    - Major option used by cells under anaerobic conditions to regenerate cytosolic NAD^+. Without O_2, mitochondria can't resupply NAD^+ - this step allows glycolysis to continue anaerobically by regenerating the NAD^+ required in step #6. Anaerobic glycolysis is obviously necessary in red blood cells, which lack mitochondria. It is also important when the oxygen supply is limiting, e.g., in exercising muscle, or anoxia due to impaired blood supply, as in stroke.
    - The microbial fermentation repertoire is vast, and includes important commercial products such as ethanol, butanol and acetone. Coelocanths, "living fossil" fish, from oxygen-poor deep sea strata, also employ alcoholic fermentation.

Summary of aerobic glycolysis:

$$\text{Glucose} + 2\ P_i + 2\ \text{ADP} + 2\ NAD^+ \rightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ H^+ + 2\ H_2O$$

Summary of anaerobic glycolysis:

$$\text{Glucose} + 2\ P_i + 2\ \text{ADP} \rightarrow 2\ \text{Lactate} + 2\ \text{ATP} + 2\ H_2O$$

Remember that phosphofructokinase 1 is inhibited by low pH. The lactate that is formed under anaerobic conditions contributes to lower blood pH. Therefore, slowing glycolysis at the committed step slows lactate production and helps prevent acidosis due to excess lactate.

Utilization of galactose:

Common dietary disaccharides are sucrose, glucose(α 1 → 2)fructose, maltose, glucose(alpha 1 → 4)glucose, and lactose, galactose(β 1 → 4)glucose. Lactase deficiency, impairing cleavage of lactose to monosaccharides, causes the digestive disorder lactose intolerance. Oxidation of galactose requires phosphorylation at carbon 1 by galactokinase followed by

Attachment to UDP by the enzyme galactose-1-phosphate uridyl transferase.

$$\text{Galactose-1-phosphate} + \text{UDP-glucose} \leftrightarrows \text{UDP-galactose} + \text{glucose-1-phosphate}$$

UDP-galactose-4-epimerase, can act:

$$\text{UDP-galactose} \leftrightarrows \text{UDP-glucose}$$

After exchange with galactose via transferase, glucose-1-phosphate is isomerized to glucose-6-phosphate by phosphoglucomutase. Genetic deficiency of transferase (galactosemia) results in inappropriate metabolism of galactose to toxic forms such as galactitol.

III. **The Tricarboxylic Acid [TCA] Cycle occurs in mitochondria.**

Each turn of the cycle produces one high energy phosphate bond in the formation of GTP (high energy phosphate transferable to ADP to form ATP) and 4 reducing equivalents (3 NADH and 1

$FADH_2$). AFTER the O_2-dependent processes of electron transport and oxidative phosphorylation, the total number of ATP produced per cycle is 10 (2.5 ATP from each NADH oxidized, 1.5 ATP from oxidation of $FADH_2$ and 1 GTP + ADP ⇆ GDP + ATP) Two molecules of pyruvate are generated from one molecules of glucose, fueling two turns of the TCA cycle. Gycolysis of 1 molecules of glucose (6 carons) yields a net of two molecules of ATP, 2 molecules of NADH (= 5 ATP) and two molecules of pyruvate (3 carbons). Each of the two molecules of pyruvate generated per glucose is converted to acetyl CoA (step # 12), producing one molecule of NADH (= 2 NADH, or 5 ATP per molecules of glucose). Acetyl CoA then enters the TCA cycle.Thus one molecule of glucose completely oxidized yields 32 ATP. Anaerobic glycolysis to lactate nets only 2 ATP per glucose molecule.

12. Formation of acetyl CoA from pyruvate by **pyruvate dehydrogenase**
    - pyruvate moves between cytosolic and mitochondrial compartments by carrier-mediated transport.
    - irreversible
    - NADH produced, CO_2 released
    - deactivated by phosphorylation by a protein kinase activity stimulated by high $NADH/NAD^+$, acetyl CoA/CoA or ATP/ADP ratios
    - pyruvate and ADP activate by inhibiting the kinase
    - dephosphorylation (by a protein phosphatase), and consequently enzymatic activity, is stimulated by insulin.
    - Ca^{2+} activates the phosphatase in heart muscle to increase energy for rapid contraction
    - competitively inhibited by acetyl CoA, NADH
    - nutrition buffs: 4 vitamins are required as co-factors: thiamin (as thiamine pyrophosphate, TPP), riboflavin (as FAD), niacin (as NAD^+) and pantothenate (as Coenzyme A). Lipoate, non-essential in the diet, is also required. Alcoholism, combined with poor nutrition, can deplete thiamin, thereby blocking the TCA cycle. For such malnourished individuals, glucose without simultaneous thiamin supplementation is harmful, because its aerobic metabolism is compromised.

13. Condensation of oxaloacetate and acetyl CoA by **citrate synthase** to form citrate
    - Irreversible
    - feed-back inhibited by citrate (the product of the enzymatic reaction)
    - ordered binding of substrates: oxaloacetate binding changes enzyme conformation to accept acetyl CoA
    - Remember, citrate is an allosteric inhibitor of reaction #3, the rate-limiting step of glycolysis catalyzed by PFKI. (Citrate can exit the mitochomdria to the cytosol, where it is converted back to acetyl CoA and oxaloacetate. The acetyl CoA provides carbon skeletons for fatty acid biosynthesis, and the oxaloacetate provides electrons for the reductive biosynthesis of fatty acids. Fatty acid biosynthesis will be discussed in the course segment on lipid metabolism.)

14. Interconversion of citrate, cis-aconitate and isocitrate by **aconitase**
    - reversible

15. Conversion of isocitrate to alpha-keto-glutarate by **isocitrate dehydrogenase**
    - irreversible oxidative decarboxylation
    - NADH produced, $C0_2$ released
    - major regulation step of the cycle responsive to the energy charge of the cell
    - the cooperative binding of isocitrate and NAD^+ is enhanced by ADP (reduced energy)
    - competitively inhibited by NADH (increased reducing power for ATP generation)
    - $Ca^{2+,}$ released from sarcoplasmic reticulum in contracting muscle lowers the K_m and speeds up the velocity of the enzyme to increase energy for muscle contraction

16. Conversion of alpha-keto-glutarate to succinyl CoA by **alpha-keto-glutarate dehydrogenase**
    - irreversible oxidative decarboxylation
    - NADH produced, CO_2 released

- product inhibited by succinyl CoA and NADH
- $Ca^{2+,}$ released from sarcoplasmic reticulum in contracting muscle lowers the K_m and speeds up the velocity of the enzyme to increase energy for muscle contraction

17. Formation of succinate from succinyl CoA by **succinyl CoA synthetase (succinate thiokinase)**
    - substrate-level phosphorylation; GTP produced

18. Conversion of succinate to fumarate by **succinate dehydrogenase**
    - $FADH_2$ produced, a C=C double bond is formed
    - Succinate dehydrogenase is a membrane-bound protein located on the inner mitochondrial membrane, unlike the other enzymes of the TCA cycle, which are located in the mitochondrial matrix.

19. Conversion of fumarate to malate by **fumarase**

20. Oxidation of malate to form oxaloacetate by **malate dehydrogenase**
    - NADH produced

Summary of the TCA cycle:

Acetyl CoA + 3 NAD^+ + FAD + GDP + P_i + 2 H_2O →

2 CO_2 + 3 NADH + $FADH_2$ + GTP + 2H^+ + CoA

Although O_2 does not participate in the TCA cycle, the cycle cannot proceed in the absence of O_2 because NAD^+ and FAD can be regenerated in the mitochondria only by the transfer of electrons from NADH and $FADH_2$ to molecular oxygen. In the absence of oxygen the concentrations of oxidized NAD^+ and FAD are diminished and NAD^+ and FAD become limiting for those reactions of the TCA cycle that require them as electron acceptors.

IV. **The Pentose Phosphate Shunt functions primarily as a source of NADPH.**

The pentose phosphate shunt (also known as the hexose monophosphate shunt or the 6-phosphogluconate pathway) shares the intermediates glyceraldehyde-3-phosphate and fructose-6-phosphate with the glycolytic pathway. Both pathways occur in the cytosol. "Cross-talk" is thus inevitable. There are two phases to the pentose phosphate shunt, the oxidative phase (reactions 21 - 23), in which NADPH and pentose-5-phosphate are generated, and the non-oxidative phase (reactions 24 - 28), in which pentose-5-phosphate can be converted to other sugars. Relative production of NADPH verses pentose sugars such as ribose varies with metabolic demand. NADPH participates in biosynthetic reductions while NADH, which is not generated by the pentose phosphate shunt, is used primarily for oxidative phosphorylation, to generate ATP. Unlike ATP and GTP, their energy charge is NOT readily exchangeable. Nucleic acid synthesis and replenishment of nucleotide cofactors require ribose.

21. Conversion of glucose-6-phosphate to 6-phosphogluconolactone by **glucose-6-phosphate dehydrogenase**
    - irreversible, rate limiting step of the pathway
    - inhibited by NADPH which competes for $NADP^+$ binding
    - NADPH produced, C=O double bond formed
    - K_m for NAD^+ is 1000-fold higher than for $NADP^+$ (In the fed state, rat liver $NADP^+$/NADPH is about 0.14, while NAD^+/NADH is about 700. This allows a high capacity for electron acceptor activity for oxidations, and at the same time, a high electron donor activity for synthesis of fatty acids and sterols.)
    - X-linked gene with mosaic expression in females

22. Conversion of 6-phosphogluconolactone to 6-phosphogluconate by **lactonase**
    - irreversible

23. Conversion of 6-phosphogluconate to ribulose-5-phosphate by **6-phosphogluconate dehydrogenase**
    - irreversible
    - NADPH produced, CO_2 released

24. Interconversion of ribulose-5-phosphate and xylulose-5-phosphate by **phosphopentose epimerase (ribulose-5-phosphate epimerase)**
    - reversible

25. Interconversion of ribulose-5-phosphate and ribose-5-phosphate by **phosphopentose isomerase (ribose-5-phosphate ketoisomerase)**
    - reversible
    - Ribose is a major component of nucleic acids.

26. Rearrangement of (ribose-5-phosphate + xylulose-5-phosphate) to form sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate by **transketolase**
    - reversible
    - Glyceraldehyde-3-phosphate is a glycolysis intermediate.
    - requires thiamine pyrophosphate [TPP]: Measurement of red blood cell transketolase activity is a convenient bioassay to test for TPP deficiency.

27. Rearrangement of sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate to form erythrose-4-phosphate and fructose-6-phosphate by **transaldolase**
    - reversible
    - Fructose-6-phosphate is a glycolysis intermediate.

28. Rearrangement of xylulose-5-phosphate and erythrose-4-phosphate to form glyceraldehyde-3-phosphate and fructose-6-phosphate by **transketolase**
    - reversible
    - Both products are also glycolysis intermediates.

In summary:

The oxidative branch of the pentose phosphate shunt can generate NADPH, without generating a net increase of ribose-phosphate, by using the ribose-phosphate sugars formed as substrates for the non-oxidative branch, which converts them into fructose-6-phosphate and glyceraldehyde-3-phosphate, which can then enter the glycolytic or gluconeogenic pathway.

$$3 \text{ glucose-6-P} + 6 \text{ NADP}^+ + 3H_2O \rightarrow 3 \text{ } CO_2 + 6 \text{ NADPH} + 6 \text{ } H^+ + 2 \text{ fructose-6-P} + \text{glyceraldehyde-3-P}$$

Alternatively, ribose-phosphate can be generated by the non-oxidative branch, without NADPH production, from the glycolytic intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate.

$$2 \text{ fructose-6-P} + \text{glyceraldehyde-3-P} \rightarrow 2 \text{ xylulose-5-P} + \text{ribose-5-P}$$

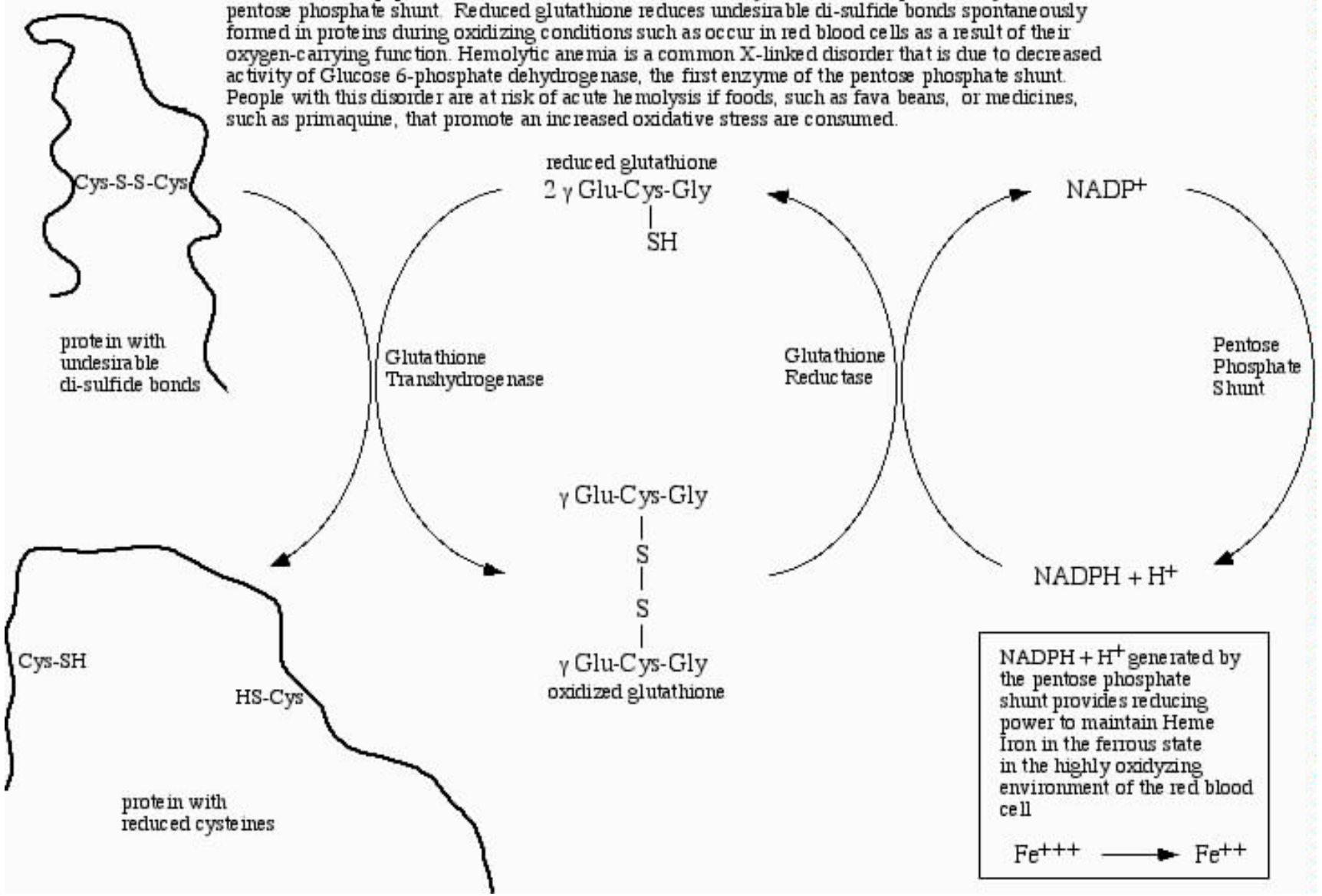
$$2 \text{ xylulose-5-P} \rightarrow 2 \text{ ribulose-5-P}$$

$$2 \text{ ribulose-5-P} \rightarrow 2 \text{ ribose-5-P}$$

The activity of the pentose phosphate shunt is low in muscle, but high in liver, testis, adrenal cortex and mammary gland where NADPH is used in the synthesis of fatty acids or steroids. Red blood cells, which are exposed to strongly oxidizing conditions as a result of their oxygen carrying function, use NADPH generated by the pentose phosphate shunt to counteract their

The pentose phosphate shunt maintains a reducing environment

Glutathione, a tripeptide, is maintained in the reduced state by NADPH + H^+ provided by the pentose phosphate shunt. Reduced glutathione reduces undesirable di-sulfide bonds spontaneously formed in proteins during oxidizing conditions such as occur in red blood cells as a result of their oxygen-carrying function. Hemolytic anemia is a common X-linked disorder that is due to decreased activity of Glucose 6-phosphate dehydrogenase, the first enzyme of the pentose phosphate shunt. People with this disorder are at risk of acute hemolysis if foods, such as fava beans, or medicines, such as primaquine, that promote an increased oxidative stress are consumed.



oxidizing environment. The major role of NADPH in red blood cells is to reduce the disulfide form of glutathione (a tripeptide of sequence *gamma*-glu-cys-gly) to the sulfhydryl form, catalyzed by glutathione reductase. The reduced glutathione serves as a "sulfhydryl buffer" to maintain the cysteine residues of hemoglobin and other red-cell proteins in the reduced state. It also serves to keep the hemoglobin iron in the ferrous state, which is crucial for hemoglobin function. Most cells (but not red blood cells which have no nuclei) require ribose-5-P for the syntheis of ribo- and deosyribonucleotides.

V. **Gluconeogenesis is NOT glycolysis run backwards**.

The daily glucose requirement for a typical adult is about 160 grams, 120 grams of which are used by the brain, which depends on a continual glucose supply. Red blood cells also require glucose as a fuel. The blood contains about 20 grams of glucose and approximately 190 grams are stored as glycogen, for a total reserve lasting about one day. During starvation glucose must be supplied from non-carbohydrate sources such as lactate, amino acids from muscle breakdown and glycerol from fat breakdown.

Six high energy bonds are expended to synthesize one molecule of glucose from 2 molecules of pyruvate. Synthesized glucose is usually for export from the liver into the blood during fasting.

29. Carboxylation of pyruvate to form oxaloacetate by **pyruvate carboxylase**
    - irreversible
    - allosteric activation by acetyl CoA
    - inhibited by ADP
    - mitochondrial localization
    - ATP is expended
    - The vitamin biotin is the required CO_2 (obtained from HCO_3-) carrier/donor
    - Oxaloacetate is transferred to the cytosol as malate, where it is regenerated by a cytosolic malate dehydrogenase.

30. Transfer of a high-energy phosphate bond to oxaloacetate to form phosphoenolpyruvate by **phosphoenolpyruvate carboxykinase**
    - inhibited by ADP
    - GTP is expended
    - transcriptionally induced by cAMP elevation during low blood glucose

31. Dephosphorylation of fructose-1,6-bisphosphate to form fructose-6-phosphate by **fructose-1,6-bisphosphatase**
    - Inhibited by fructose-6-phosphate, AMP and fructose-2,6-bisphosphate
    - Stimulated by citrate

32. Dephosphorylation of glucose-6-phosphate to form glucose by **glucose-6-phosphatase**
    - membrane-bound in the endoplasmic reticulum
    - This enzyme is present only in liver and kidney, the two tissues that can export glucose into the blood.
    - Recent evidence indicates that transcription of the glucose-6-phosphatase gene is up regulated by cyclic AMP (cAMP) and down regulated by insulin. Therefore, when blood glucose is low, glucagon increases and signals to cause an increase of cAMP in the liver. cAMP activates CREB (cAMP response element binding protein), which activates glucose-6-phosphatase gene transcription, resulting in increased glucos-6-phosphatase enzyme and increased conversion of glucose-6-phosphate to glucose for export to the blood to maintain blood glucose homeostasis. Insulin, which increases in response to increased blood glucose, causes suppression of glucose-6-phosphatase gene transcription, thereby down regulating export of glucose to the blood by the liver when blood glucose increases.
    - Recall that glucokinase gene transcription is repressed by glucagon (cAMP) in response to low blood glucose levels. Also, when blood glucose levels are low existing glucokinase enzyme is bound by the Glucokinase Regulatory Protein [GKRP], which inactivates and

sequesters it in the cell nucleus, thereby preventing the glucose produced by gluconeogenesis and glycogenolysis from being re-phosphorylated and retained in the liver. The glucose produced in the liver is, therefore, destined for export to the blood. Glucokinase gene transcription is stimulated by insulin which is increased when blood glucose levels increase. The up regulation of glucose-6-phosphatase and the down regulation of glucokinase are adaptive to maintain blood glucose homeostasis to ensure that tissues that require glucose have it available.

Summary of gluconeogenesis:

$$2 \text{ Pyruvate} + 4 \text{ ATP} + 2 \text{ GTP} + 2 \text{ NADH} + 4 \text{ } H_2O \rightarrow$$

$$\text{Glucose} + 4 \text{ ADP} + 2 \text{ GDP} + 6 \text{ } P_i + 2 \text{ } NAD^+ + 2H^+$$

The Cori cycle:

In the starved state liver synthesizes glucose (or releases glucose from its glycogen stores - see below) and furnishes it to other tissues. Exercising skeletal muscle or red blood cells derive energy from glucose by glycolysis. The resulting pyruvate is converted to lactate, thereby regenerating NAD^+ from the NADH produced in step #6, so that glycolysis can continue to supply energy. Muscle or red blood cells release the lactate into the blood from which it is absorbed by the liver, which converts it, by gluconeogenesis, back to glucose, to be re-supplied to other tissues as needed.

VI. **Glycogen Metabolism plays different roles in muscle and liver.**

The liver is a so-called "altruistic" organ, which releases glucose into the blood to meet the needs of working muscle or brain. Glucose released from muscle glycogen stores is used on site to provide energy for muscle contraction. Like glycolysis and gluconeogenesis, glycogenolysis and glycogenesis are NOT reversals of each other.

33. Interconversion of glucose-6-phosphate and glucose-1-phosphate by **phosphoglucomutase**
    - reversible - this is the only enzyme common to both glycogen synthesis and breakdown

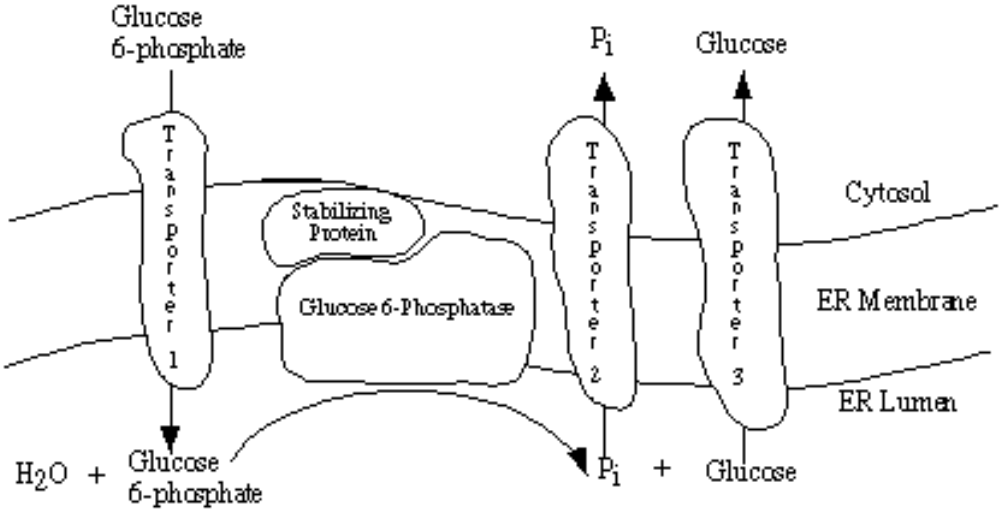
34. Activation of glucose-1-phosphate to form UDP-glucose by **UDPG pyrophosphorylase**

35. Transfer of glucose from UDP-glucose to glycogen by **glycogen synthase**
    - does not initiate new molecules of glycogen but requires a "primer" at least 4 glucose units long
    - Glycogenin, a protein, autocatalyzes the synthesis of a glycogen primer, covalently attached to itself, which glycogen synthase extends. Therefore, the number of molecules of glycogenin present determines the number of glycogen molecules synthesized.
    - Glycogen synthase must always be in contact with glycogenin to be active. Therefore, the size of a glycogen molecule is limited by the physical distance between its most distal non-reducing end and glycogenin, which is covalently attached to its reducing end. When this distance is longer than glycogen synthase can span elongation of the glycogen molecule halts.
    - inactivated by phosphorylation via cAMP-dependent cascade
    - inactivation reversed by insulin, which stimulates dephosphorylation

36. Transfer of $(\text{glucose})_{>6}$ from alpha-1,4 glycosidic linkage to 1,6 linkage by **branching enzyme**

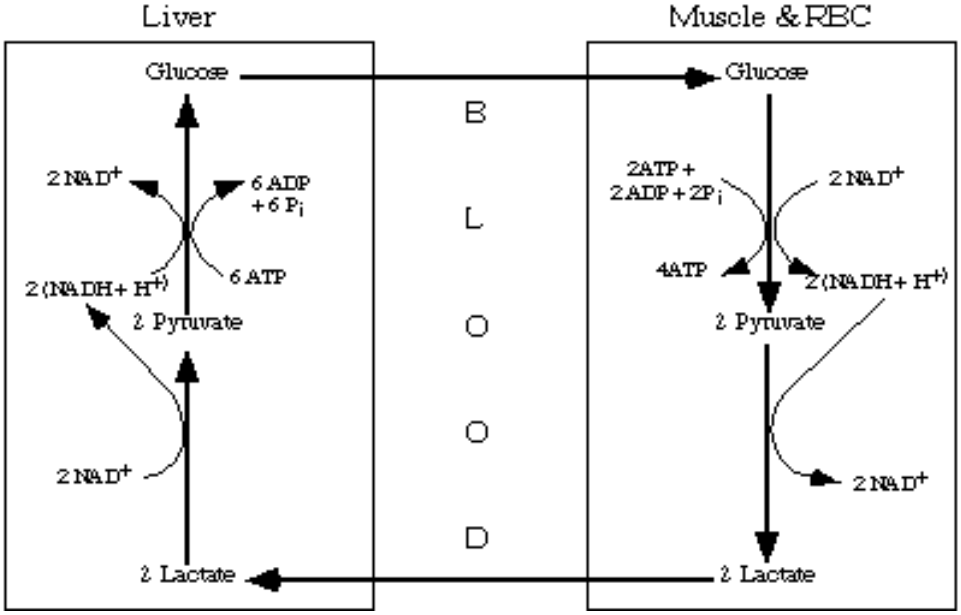
Glucose is generated from Glucose 6-Phosphate in the lumen of the Endoplasmic Reticulum

Glucose 6-Phosphatase activity is present at the inner surface of the endoplasmic reticulum [ER] in liver, but not in other tissues, with the exception of kidney. Glucose 6-phosphate is transported into the ER lumen by a transporter protein [transporter 1]. Glucose 6-Phosphatase catalyses hydrolyses of the phosphate, yielding inorganic phosphate [P_i], which is transported out of the ER lumen to the cytosol by a second transporter protein [transporter 2], and glucose, which is transported out of the ER lumen to the cytosol by a third transporter protein [transporter 3]. An essential stabilizing protein is associated with Glucose 6-Phosphatase. The glucose produced by Glucose 6-Posphatase does not become rephosphorylated by Glucokinase because when glucagon is high the gene for Glucokinase is not transcribed, thereby reducing the amount of Glucokinase protein synthesized. Also, the existing Glucokinase is bound by Glucokinase Regulatory Protein [GKRP], which sequesters it in the cell nucleus, both inactivating it and separating it from the glucose produced by Glucose 6-Phosphatase.



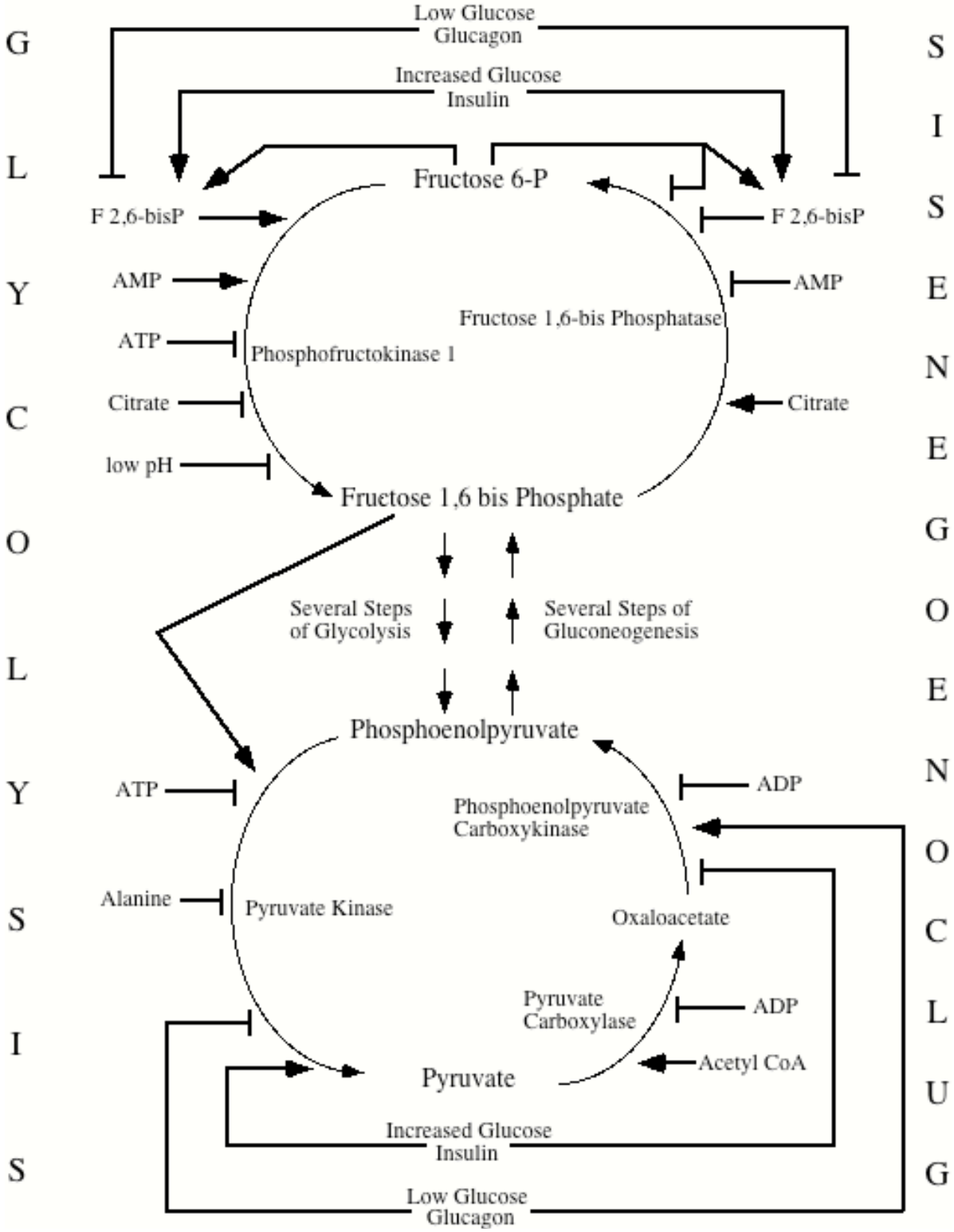
The Cori Cycle

Shifting the metabolic burden of regenerating NAD^+ from active skeletal muscle and red blood cells to the liver



Regulation of Glycolysis and Gluconeogenesis

Glycolysis and gluconeogenesis are biochemical pathways that, respectively, break down and synthesize glucose. Gluconeogenesis occurs in the liver to supply glucose for export to the blood during times of low blood glucose levels. NOTE the reciprocal regulation of glycolysis and gluconeogenesis which avoids a futile cycle of simultaneous glucose break down and synthesis.



- more compact storage, more accessible free ends for synthesis and phosphorylase (see below)
- The block transferred is at least 7 residues long, must include the non-reducing end, must come from a chain of at least 11 residues long (thereby leaving a primer of at least 4 residues as is required by glycogen synthase) and is transferred at least 4 residues away from a pre-existing branch.

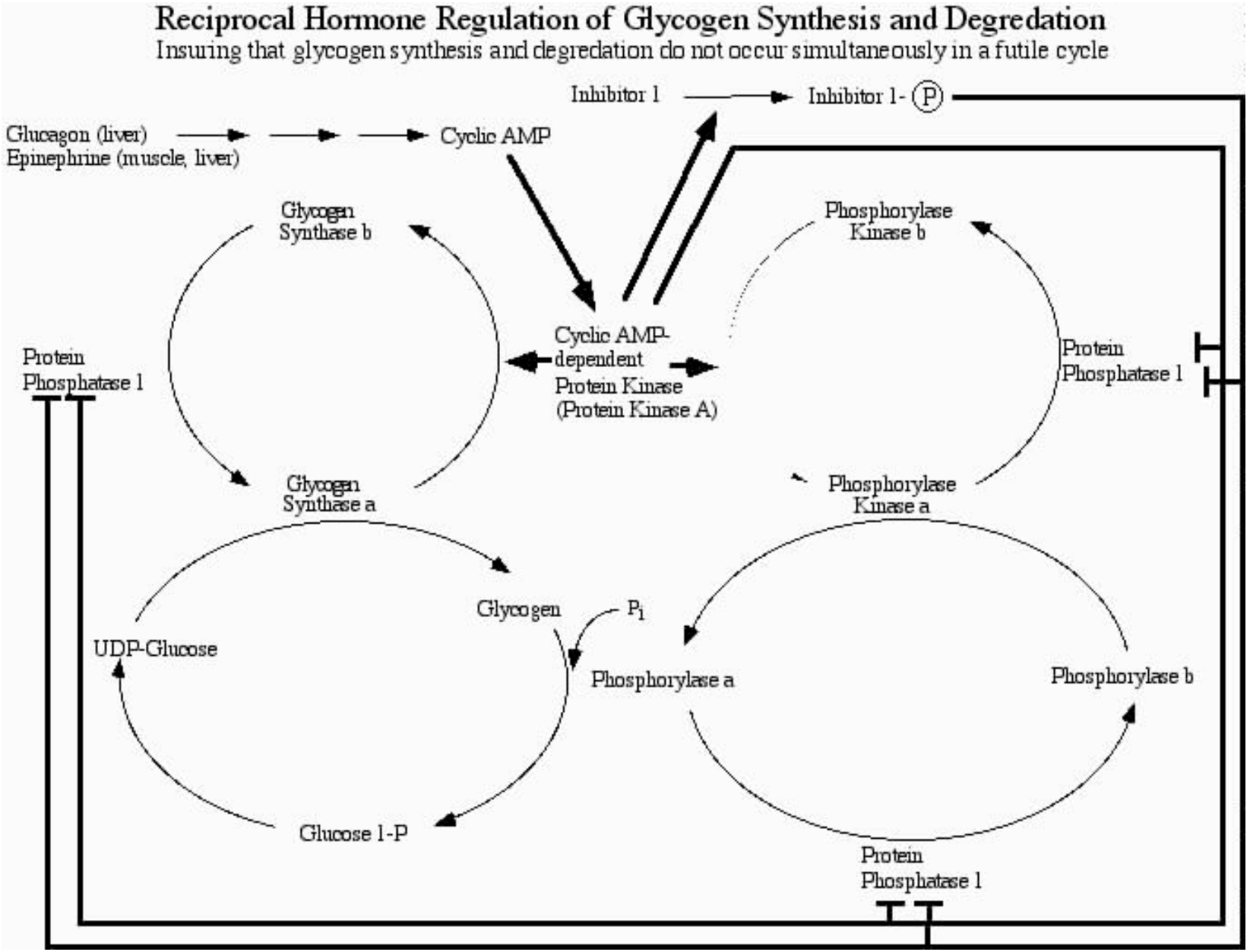
Summary of glycogen synthesis:

$$\text{Glucose-6-phosphate} + \text{ATP} + \text{glycogen}_n + H_2O \rightarrow \text{glycogen}_{n+1} + \text{ADP} + 2\ P_i$$

37. Phosphorolysis of alpha-1,4-glycosidic bonds of glycogen to release glucose-1-phosphate sequentially from the non-reducing end by **glycogen phosphorylase**
    - In vivo, [P_i] is about 100-fold higher than [glucose-1-phosphate], preventing reversal
    - The muscle and liver phosphorylase isoforms are distinct.
    - Glucagon (liver) or epinephrine (muscle and liver) triggered cAMP-dependent phosphorylation cascade results in the phosphorylation of serine 14, switching the enzyme to the activated,"*a* " form.
        - — Phosphorylation of glycogen phosphorylase requires a second protein kinase. cAMP-dependent protein kinase (protein kinase A) phosphorylates phosphorylase kinase (the second protein kinase), converting it from the inactive "*b* " form to the active "*a* " form. Phosphorylase kinase then phosphorylates glycogen phosphorylase at serine 14, converting the inactive "*b* " form to the active "*a* " form.
    - Glucose-binding causes a conformation change in the liver "*a* " isoform, exposing the phosphoserine to protein phosphatase 1, regenerating the inactive phosphorylase "*b* " form. Thus, in liver, glycogen breakdown by glycogen phosphorylase is regulated by the glucose concentration, consistent with the function of the liver to provide glucose to other tissues.
    - The muscle isoform of phosphorylase *b* can be activated by binding AMP, which is present in high concentrations when the energy charge of the cell is low. ATP and glucose-6-phosphate reverse the activation by AMP by competing for the AMP binding site. Thus, in muscle, glycogen breakdown catalyzed by glycogen phosphorylase is regulated by the energy charge of the cell, consistent with the function of muscle glycogen, to act as an energy store to be mobilized to supply energy for muscle contraction. The liver isoform of phosphorylase *b* is not activated by AMP.

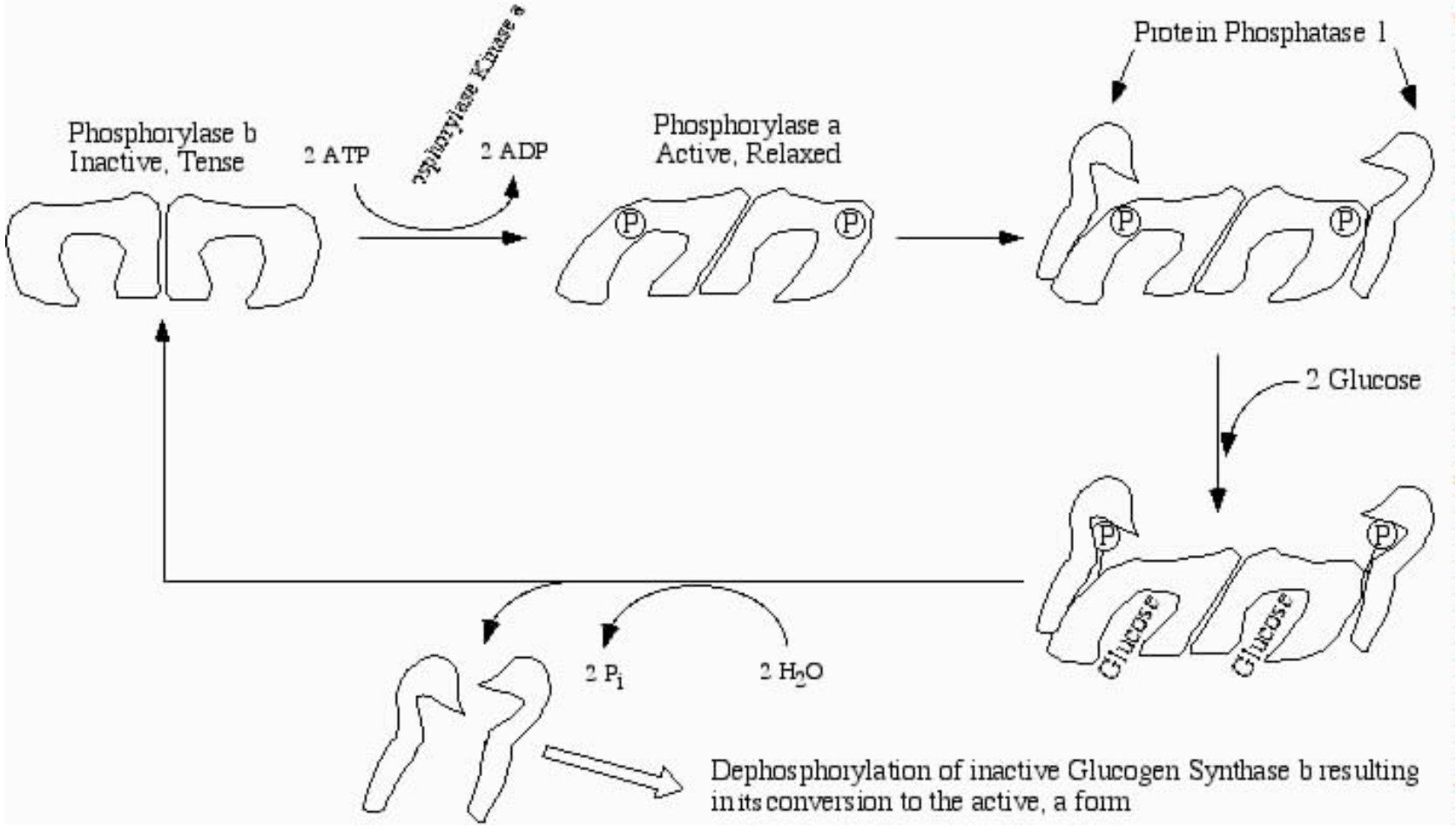
38. Hydrolysis of glycogen branchpoints by **transferase** and **alpha-1,6-glucosidase (debranching enzyme)** to release glucose
    - two step reaction, both activities in one protein

39. Phosphorylation of glucose to form glucose-6-phosphate by **hexokinase** (muscle) or **glucokinase** (liver) and **phosphoglucomutase** (steps #1 and #33)


Reciprocal Hormone Regulation of Glycogen Synthesis and Degredation
Insuring that glycogen synthesis and degredation do not occur simultaneously in a futile cycle
Inhibitor 1
Inhibitor 1- P
Glucagon (liver)
Epinephrine (muscle, liver)
Cyclic AMP
Glycogen Synthase b
Phosphorylase Kinase b
Protein Phosphatase 1
Cyclic AMP-dependent Protein Kinase (Protein Kinase A)
Protein Phosphatase 1
Glycogen Synthase a
Phosphorylase Kinase a
Glycogen
P_i
UDP-Glucose
Phosphorylase a
Phosphorylase b
Glucose 1-P
Protein Phosphatase 1

Glycogen Phosphorylase regulation by Glucose in the Liver

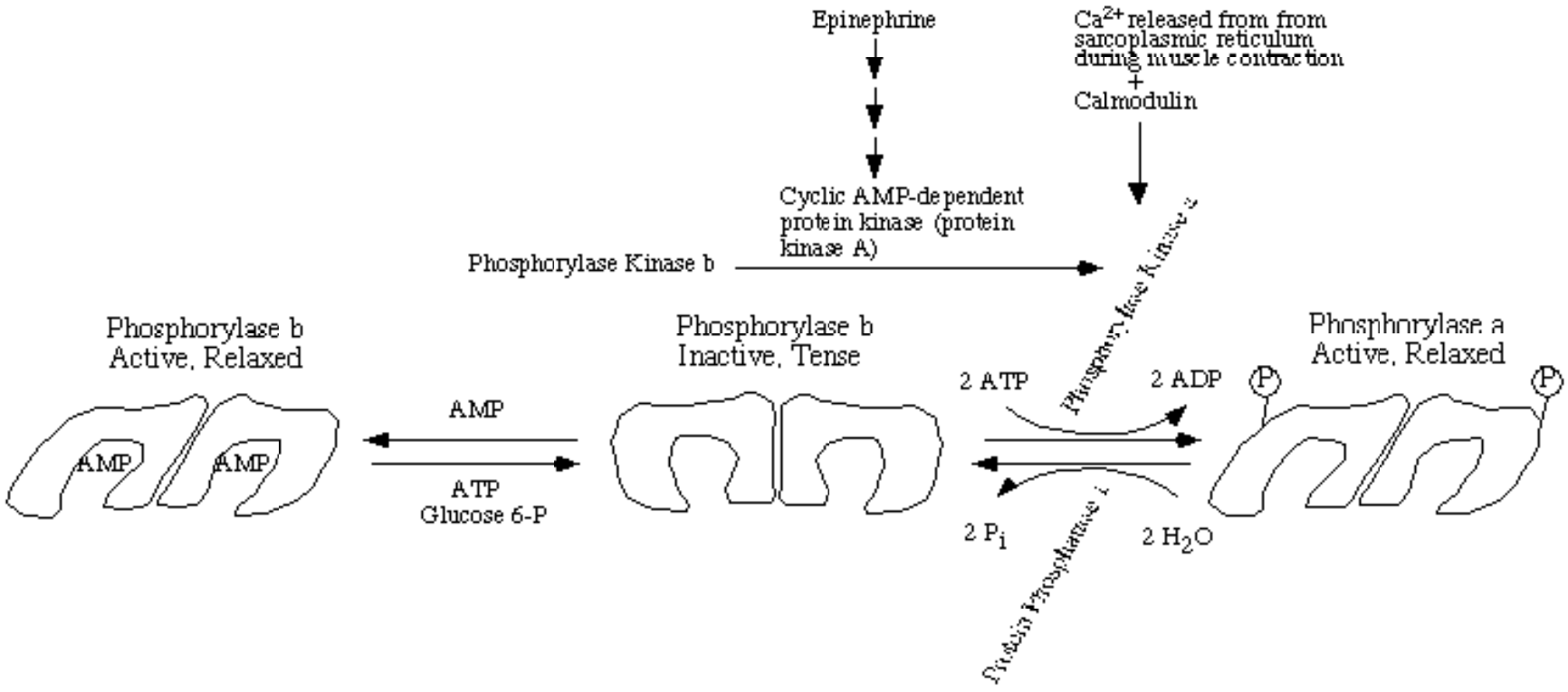
The function of liver glycogen is to supply glucose for export to the blood when blood glucose levels fall. In keeping with this function, as glucose levels rise in the liver, glucose binding to active glycogen phosphorylase (the a form) causes a conformational change in the enzyme, exposing the phosphate groups to the activity of protein phosphatase 1, which binds only to the a form of glycogen phosphorylase, and cleaves the phosphates, thereby converting the active phosphorylase a form to the inactive phosphorylase b form. Protein phosphatase 1 does not bind to the inactive b form of glycogen phosphorylase and is released so it can de-phosphorylate glycogen synthase b to convert it to the active, a form. Thus, only after glycogen phosphorylase has been converted to the inactive b form is inactive glycogen synthase b converted to the active a form, thereby preventing a futile cycle of simultaneous glycogen degradation and synthesis.



Regulation of Muscle Glycogen Phosphorylase by the small molecules AMP, ATP, Glucose 6-P, Ca^{2+} and by the hormone Epinephrine

The function of muscle glycogen is to serve as a store of glucose to be used, on site, as a source of energy for muscle activity. Muscle does not export glucose to the blood. In keeping with the function of glycogen degradation in the muscle, muscle glycogen phosphorylase is regulated by small molecules that serve as indicators of the energy level of the muscle cell, namely, AMP, ATP and glucose 6-phosphate.

The inactive, b form of muscle glycogen phosphorylase be can be activated by two mechanisms. Phosphorylation by cyclic AMP-dependent protein kinase, generated in response to activation of the epinephrine signal transduction pathway, converts inactive muscle phosphorylase b to active phosphorylase a. This pathway serves to activate glycogen breakdown in muscles in response to increased levels of epinephrine (adrenaline) in the blood. The resulting increase in muscle glucose serves as a source of muscle energy to deal with emergency (fight-or-flight) situations.
Glycogen breakdown for energy for routine muscle activity is regulated by the energy charge of muscle cells. The inactive b form of muscle glycogen phorphorylase can be activated by binding AMP, increased levels of which are indicative of a low cellular energy charge. The binding of AMP is reversed when ATP levels increase (causing a decrease in the AMP / ATP ratio). Likewise, glucose 6-phosphate also reverses the activation of muscle glycogen phosphorylase by AMP. NOTE that AMP does not bind to liver glycogen phosphorylase and it is not regulated by cellular energy levels, but rather by glucose levels, in keeping with its function in the production of glucose by the liver for export to the blood during times of low blood glucose levels. Glucose is not a regulator of muscle glycogen phosphorylase. Ca^{2+} released from the muscle sarcoplasmic reticulum in response to neural impulses that initiate contraction bind and activate the protein calmodulin, which is a modifier protein that activates phosphorylase kinase.



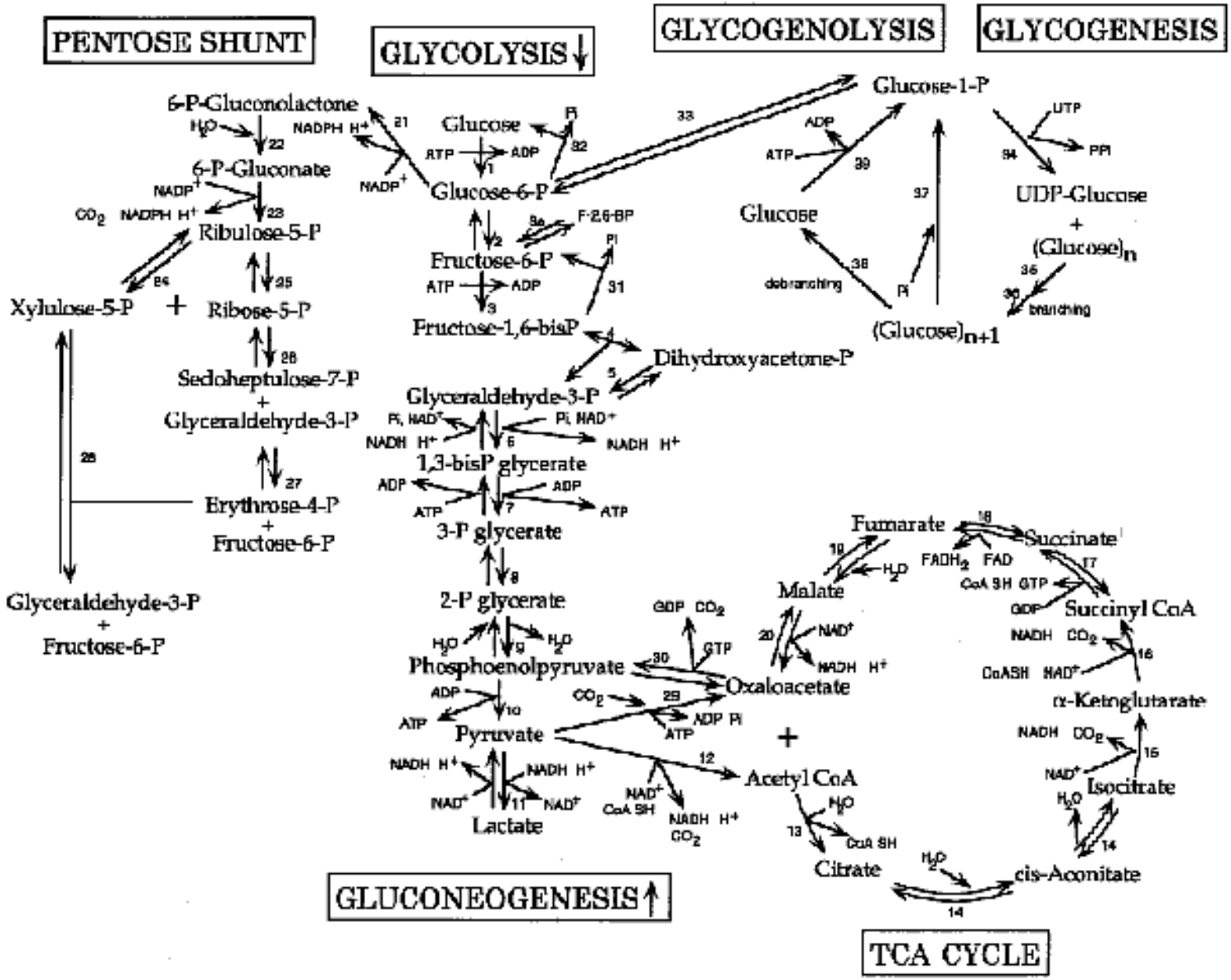
Protein phosphatase 1 reverses the regulatory effects of protein kinases on glycogen metabolism.

Protein phosphatase 1 removes the phosphate groups from phosphorylase kinase, glycogen phosphorylase and glycogen synthase. Glucagon- (liver) or epienphrine- (liver and muscle) activated protein phosphorylation inactivates protein phosphatase 1, thereby preventing it from removing phosphate groups from phosphorylase kinase, glycogen phosphorylase and glycogen synthase. In addition, an inhibitor of protein phosphatase 1, inhibitor 1, when phosphorylated by cAMP-dependent protein kinase (protein kinase A) also inhibits protein phosphatase 1. Insulin activates protein phosphatase 1. The resulting desphosphorylation of phosphorylase kinase, glycogen phosphorylase and glycogen synthase inhibits glycogen breakdown and promotes glycogen synthesis. In the liver, protein phosphatase 1 binds to glycogen phosphorylse, *a* but in the absence of glucose glycogen phosphorylase *a* is refractory to the action of protein phosphatase 1. When glucose accumulates (e.g., as a result of dietary glucose intake) it binds to, and causes a conformational change in glycogen phosphorylase *a* exposing serine 14, which becomes sensitive to protein phosphatase 1. Protein phosphatase 1 cleaves the phosphate group from serine 14, thereby converting phosphorylase *a* to phosphorylase *b* and slowing glycogenolysis. Protein phosphatase 1 does not bind to glycogen phosphorylase *b* and it is free to dephosphorylate, and thereby activate, glycogen synthase. This reciprocal regulatory mechanism depends on (1) communication between the serine phosphate of glycogen phosphorylase and the allosteric site for glucose. (2) the use of protein phosphatase 1 to inactivate glycogen phosphorylase and activate glycogen synthase, (3) the binding of protein phosphatase 1 to phosphorylase *a* , to prevent it from activating glycogen synthase, but not to phosphorylase *b*. This mechanism also depends on the number of glycogen phosphorylase *a* molecules being in excess of the number of protein phosphatase 1 molecules so that all the protein phosphatase 1 molecules are bound by glycogen phosphorylase *a* , a condition which is achieved by the initial ratio of glycogen phosphorylase *a* / protein phosphatase 1 = 10 / 1.

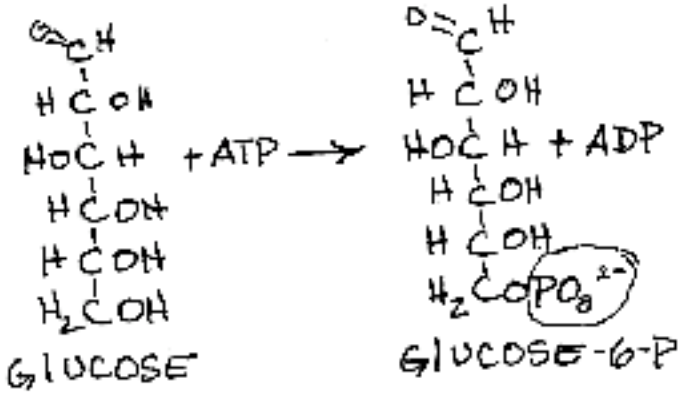
Glycogen storage diseases:

Imbalance between glycogenolysis and glycogenesis, or between branching and debranching activities results in storage of abnormal amounts of glycogen or of structurally abnormal glycogen, which can cause serious impairment of cell and organ functions. Human genetic defects in enzymes #3, #32 and #36-38 cause glycogen storage diseases. You should now be able to figure out for each mutant enzyme whether glycogen amount, structure, or both is affected. You should also consider, for enzymes with tissue-specific isoforms, which organs may be affected. In addition to the enzymes described above, all cells contain a lysosomal enzyme, alpha-1,4-glycosidase. Mutations in its gene have global effects.

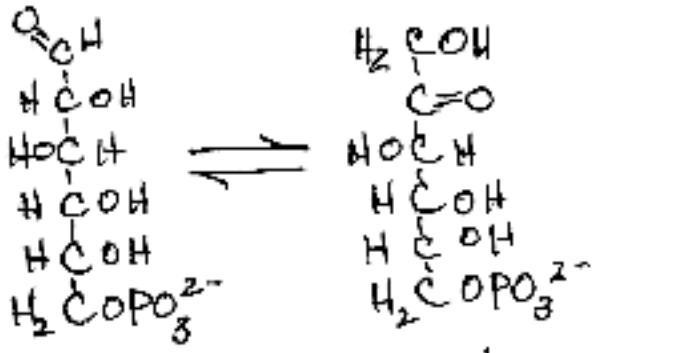
What consequences do you expect from a defect in phosphorylase kinase?


PENTOSE SHUNT
GLYCOLYSIS
GLYCOGENOLYSIS
GLYCOGENESIS
6-P-Gluconolactone
6-P-Gluconate
Ribulose-5-P
Xylulose-5-P
Ribose-5-P
Sedoheptulose-7-P
Glyceraldehyde-3-P
Erythrose-4-P
Fructose-6-P
Glucose
Glucose-6-P
Fructose-6-P
Fructose-1,6-bisP
F-2,6-BP
Dihydroxyacetone-P
Glyceraldehyde-3-P
1,3-bisP glycerate
3-P glycerate
2-P glycerate
Phosphoenolpyruvate
Pyruvate
Lactate
Acetyl CoA
Citrate
cis-Aconitate
Isocitrate
α-Ketoglutarate
Succinyl CoA
Succinate
Fumarate
Malate
Oxaloacetate
Glucose-1-P
UDP-Glucose
(Glucose)n
(Glucose)n+1
GLUCONEOGENESIS
TCA CYCLE

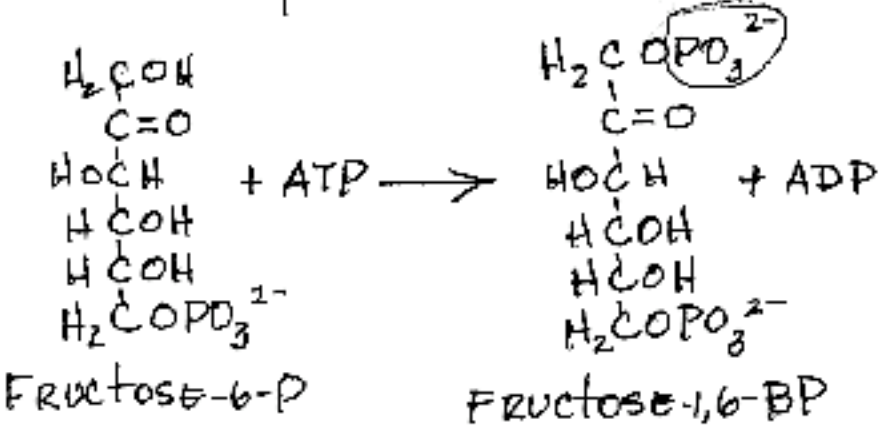
No 1: HEXOKINASE



No 2: Phosphoglucose Isomerase

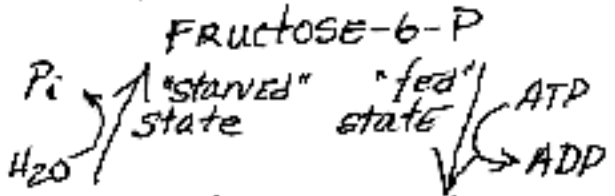


No 3: PhosphofructoKINASE I



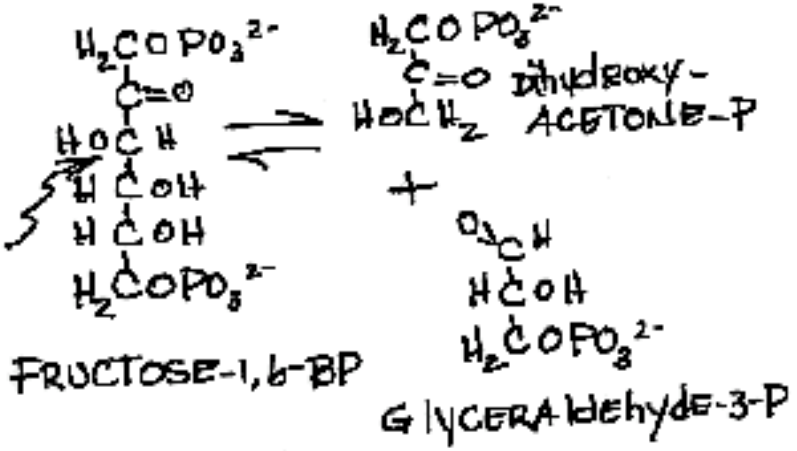
No 3a: 1 protein w/ 2 Active sites!

PhosphofructoKINASE II

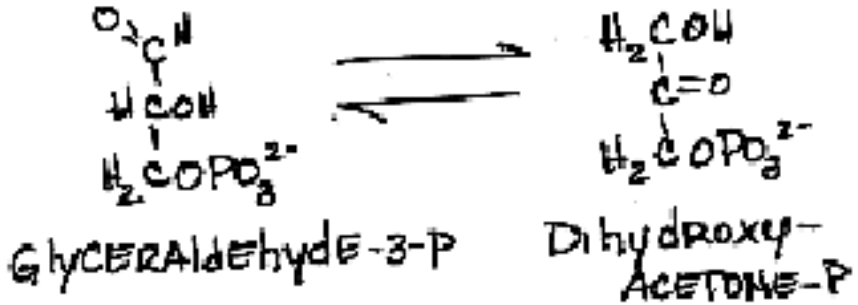


FRUCTOSEbisphosphATASE II

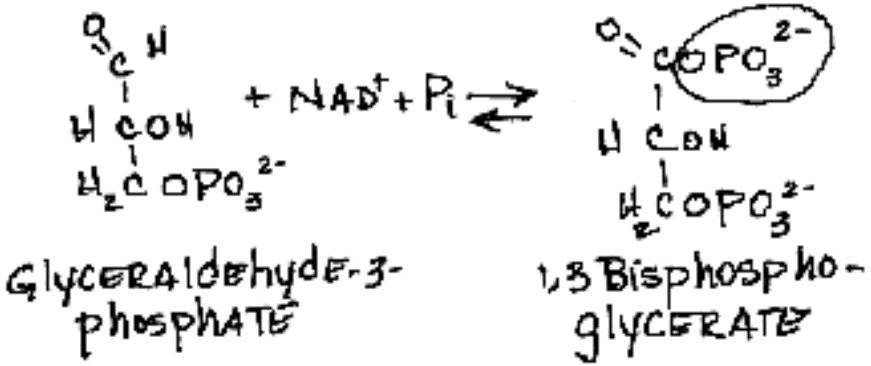
No 4: Aldolase



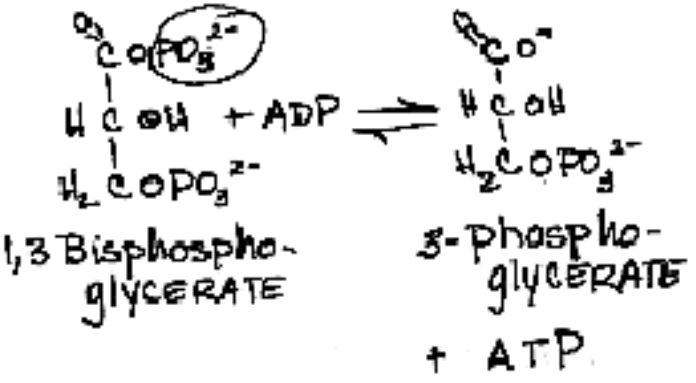
No 5: Triose Phosphate Isomerase



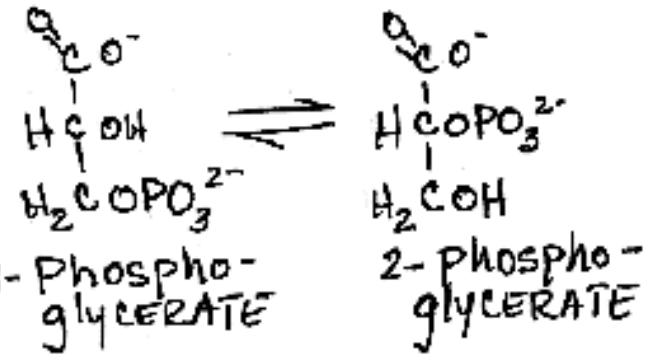
No 6: Glyceraldehyde-3-phosphate Dehydrogenase



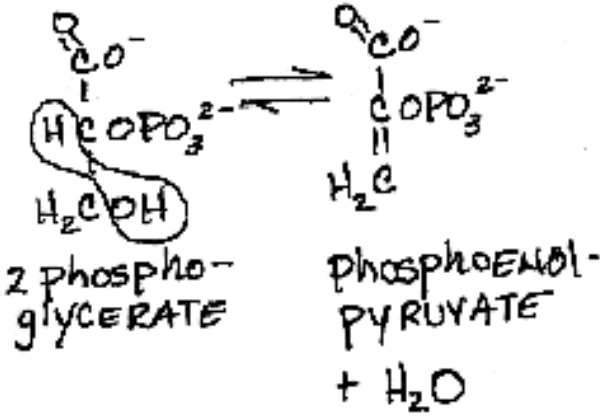
No 7: Phosphoglycerate Kinase



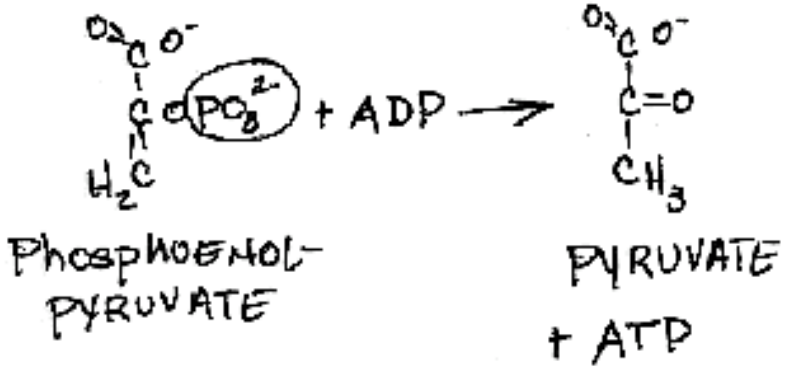
No 8: Phosphoglycerate Mutase



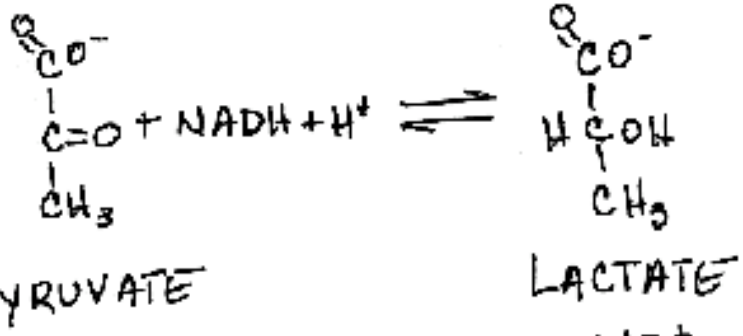
No 9: Enolase



No 10: Pyruvate Kinase



No 11: Lactate Dehydrogenase



EATING Milk

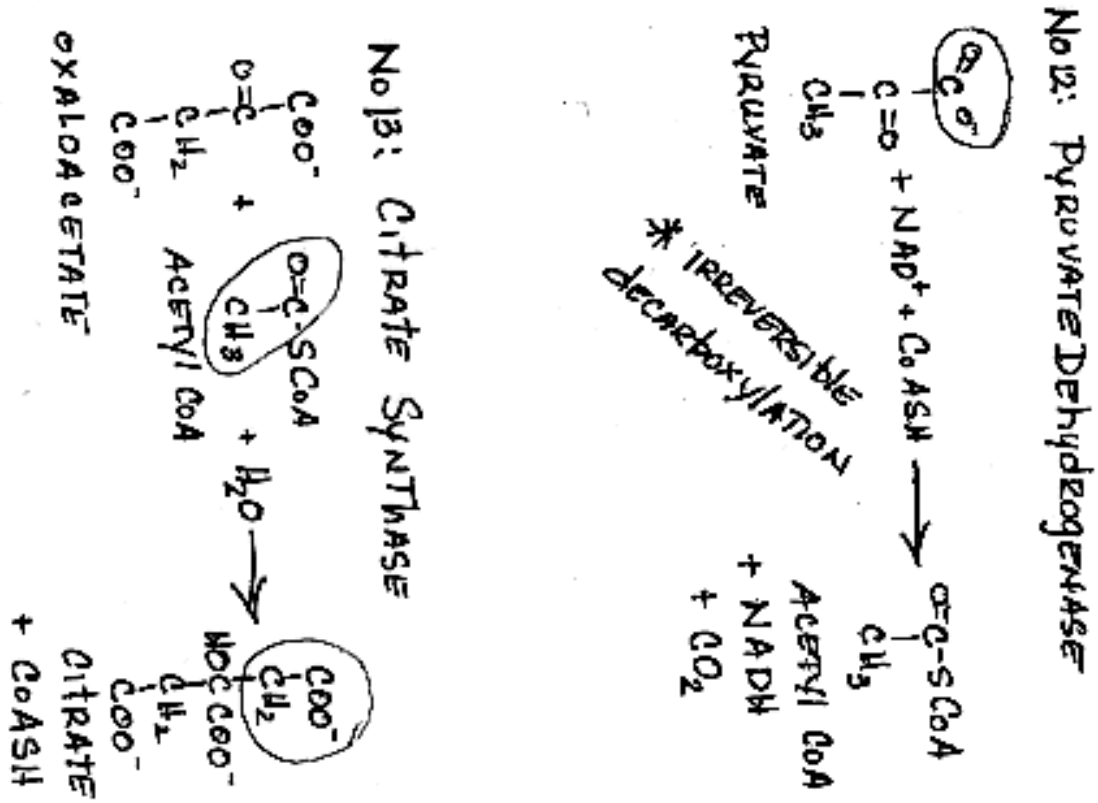
GAL β(1-4) GLUC —LACTASE→ GAL + GLUC
LACTOSE

GAL + ATP —GALACTOKINASE→ GAL-1-P + ADP

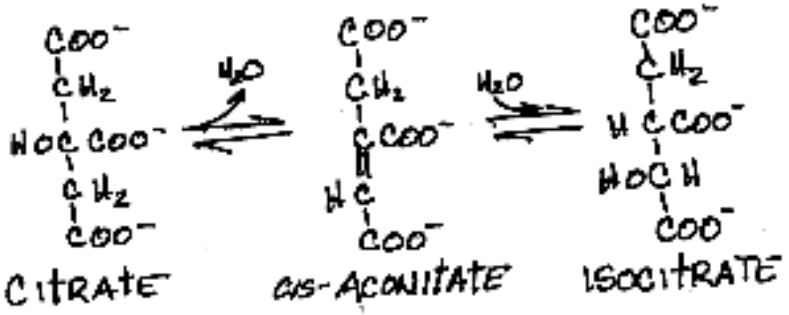
GAL-1-P + UDP-GLUC ⇌ UDP-GAL + GLUC-1-P
(GALACTOSE-1-P URIDYL TRANSFERASE)

UDP-GAL ⇌ UDP-GLUC
(UDP-GALACTOSE 4-EPIMERASE)

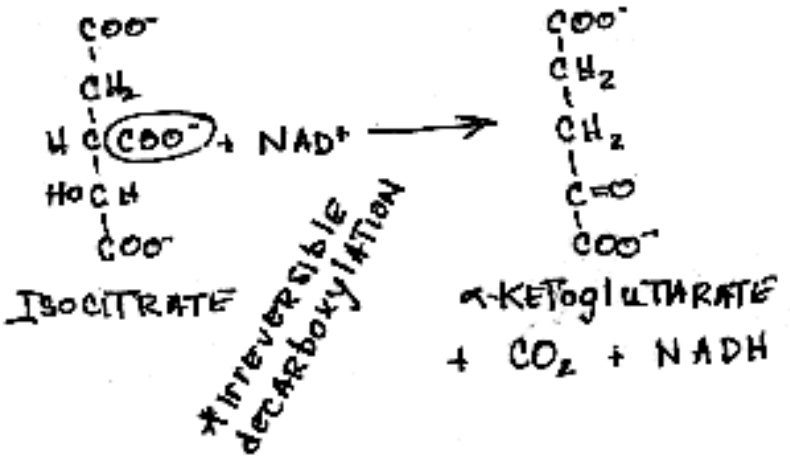
GLUC-1-P ⇌ GLUC-6-P
(PHOSPHOGLUCOMUTASE)



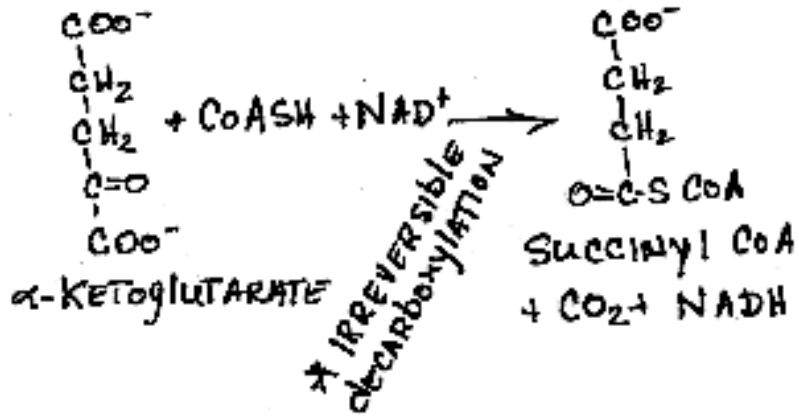
No 14: Aconitase



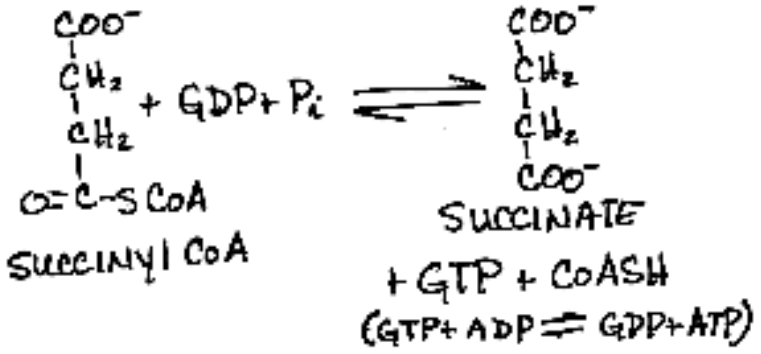
No 15: Isocitrate Dehydrogenase



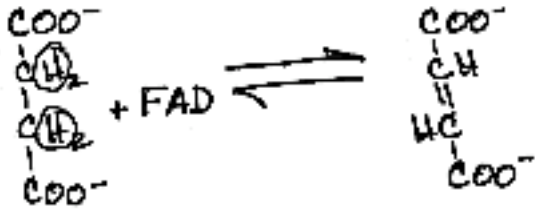
No 16: α-Ketoglutarate Dehydrogenase



No 17: Succinyl CoA Synthetase

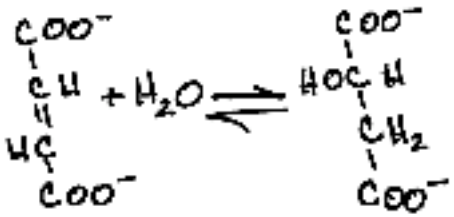


No 18: Succinate Dehydrogenase



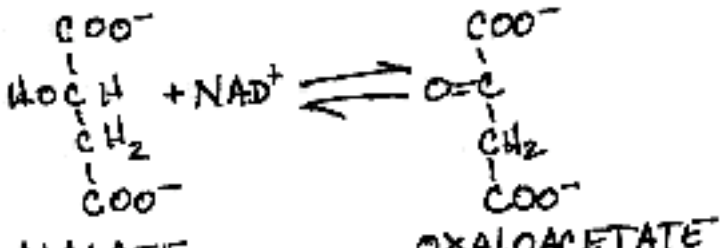
Succinate → Fumarate + $FADH_2$

No 19: Fumarase



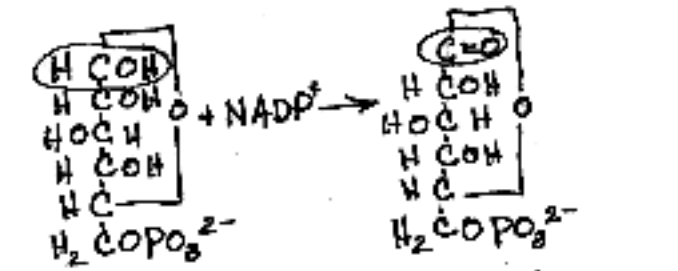
Fumarate → Malate

No 20: Malate Dehydrogenase



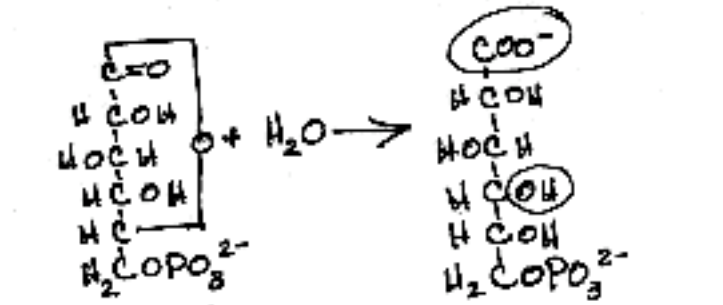
Malate → Oxaloacetate + $NADH + H^+$

No 21: Glucose-6-Phosphate Dehydrogenase



Glucose-6-P → 6 Phosphogluconō-δ-lactone + $NADPH + H^+$

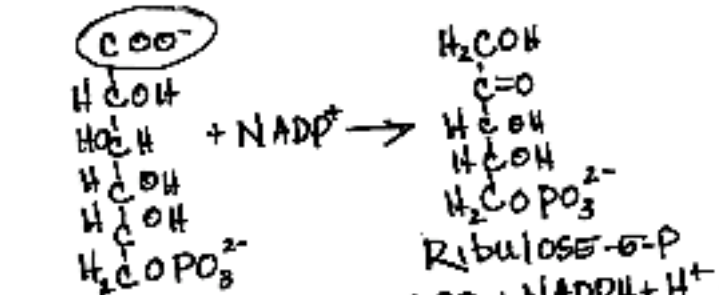
No 22: LACTONASE



6 phosphoglucono-δ-lactone

6 phosphogluconate + H^+

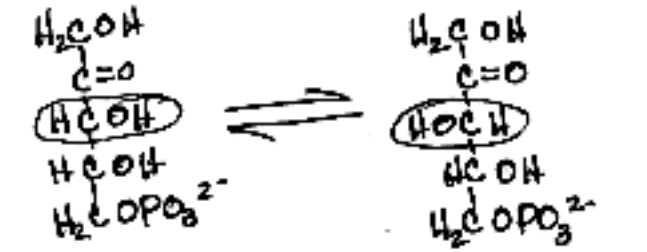
No 23: 6-Phosphogluconate Dehydrogenase



6-phosphogluconate

Ribulose-5-P + CO_2 + NADPH + H^+

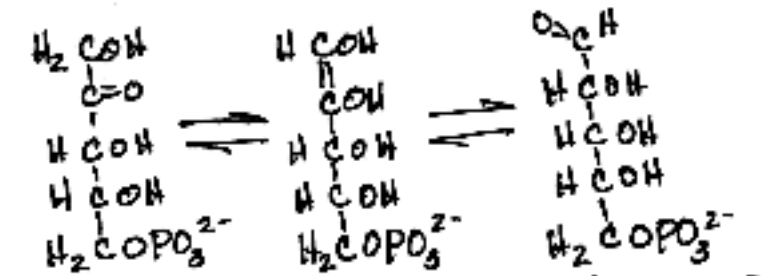
No 24: Phosphopentose Epimerase



Ribulose-5-P

xylulose-5-P

No 25: Phosphopentose Isomerase

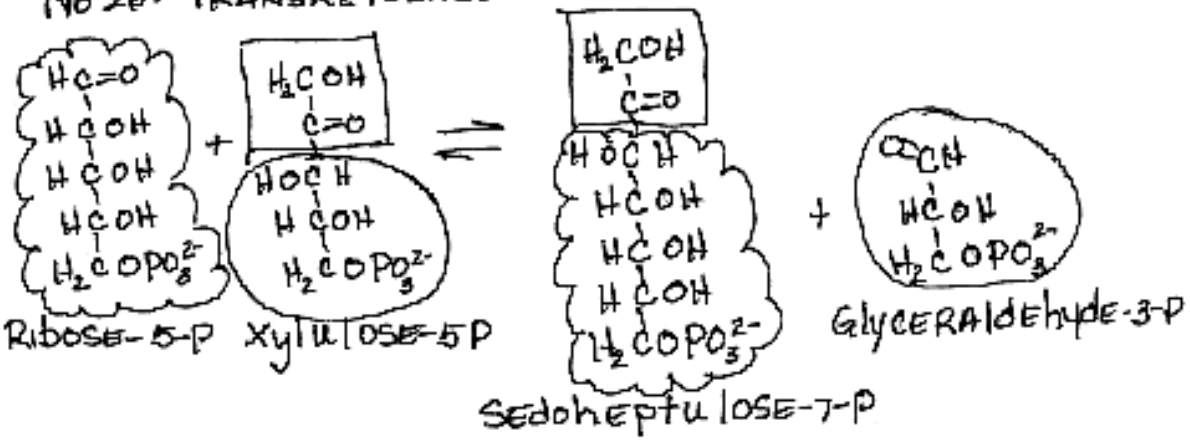


Ribulose-5-P

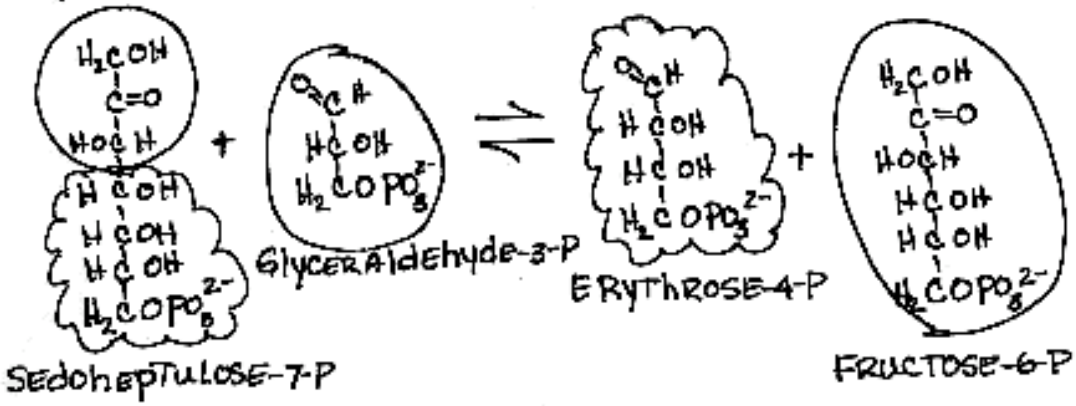
Enediol intermediate

Ribose-5-P

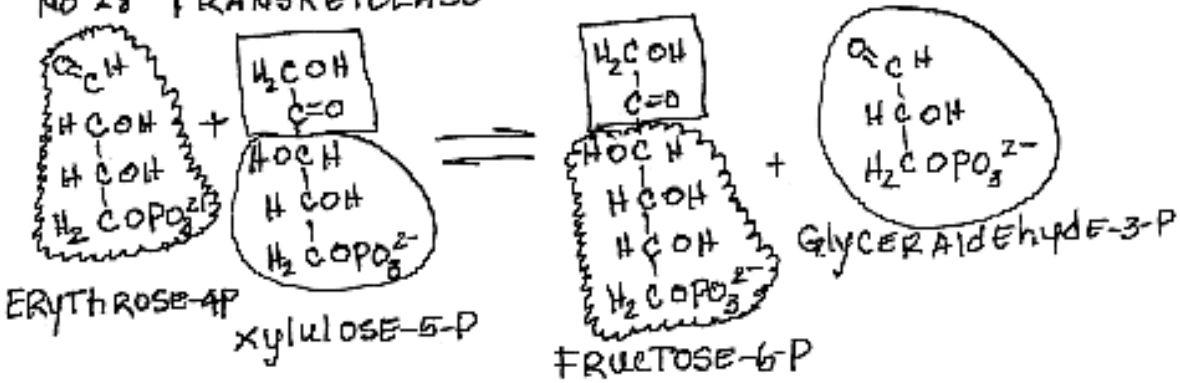
No 26: TRANSKETOLASE



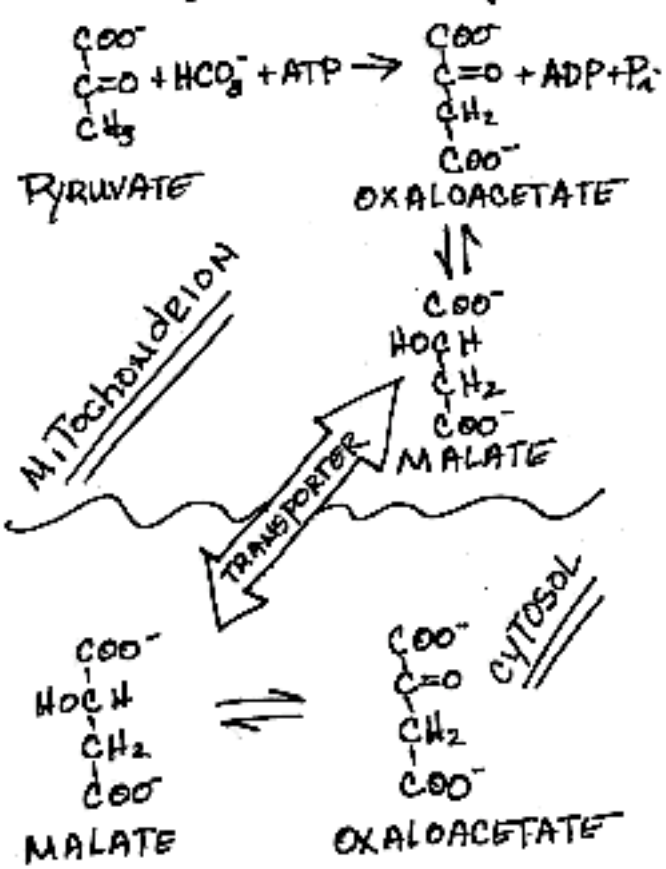
No 27: TRANSALDOLASE



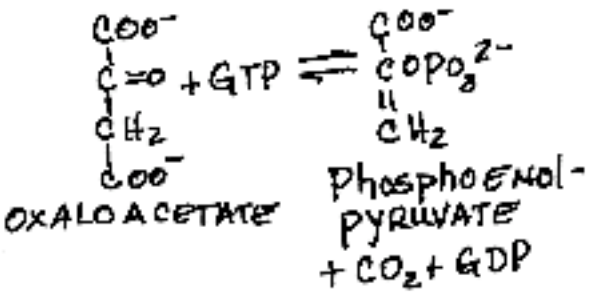
No 28 TRANSKETOLASE



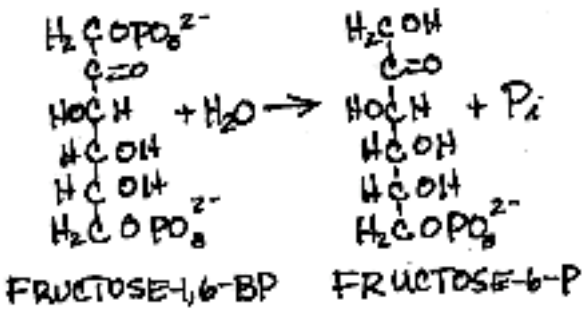
No 29: PYRUVATE CARBOXYLASE



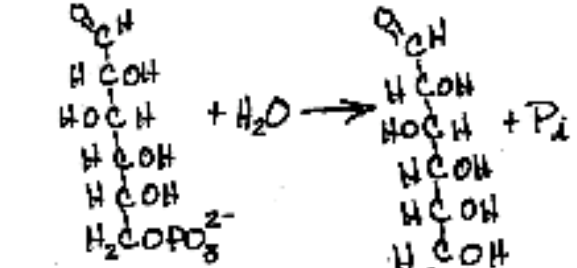
No 30: PHOSPHOENOLPYRUVATE CARBOXYKINASE



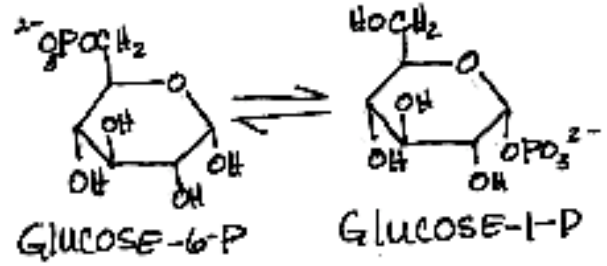
No 31: FRUCTOSE-1,6-BISPHOSPHATASE



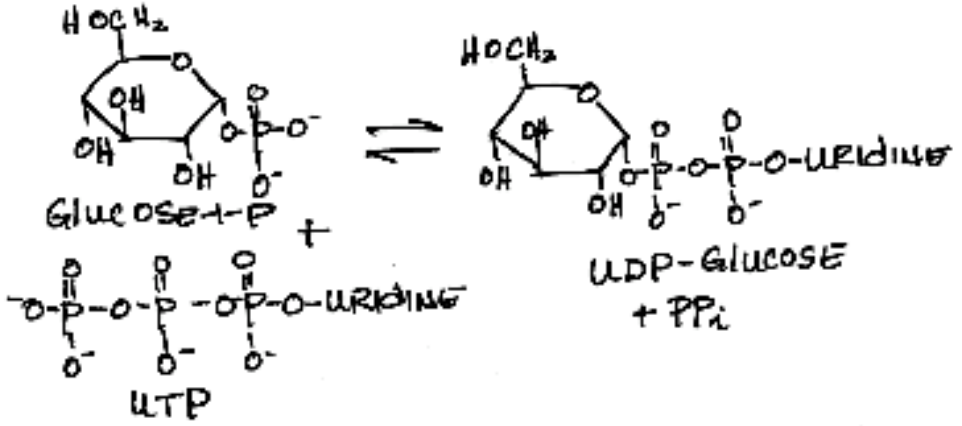
No 32: Glucose-6-phosphatase
IN THE ENDOPLASMIC RETICULUM



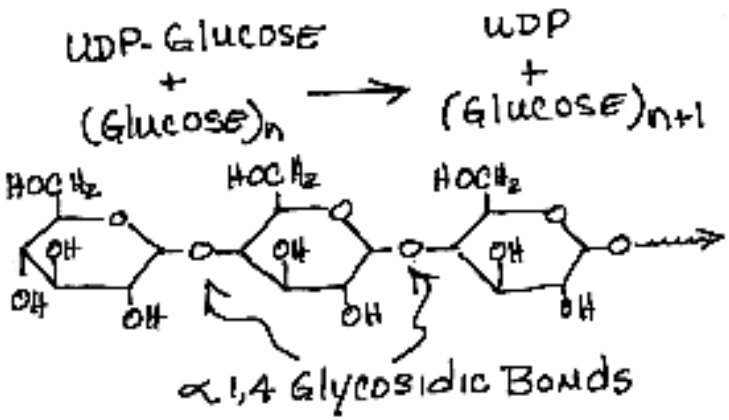
No 33: Phosphoglucomutase



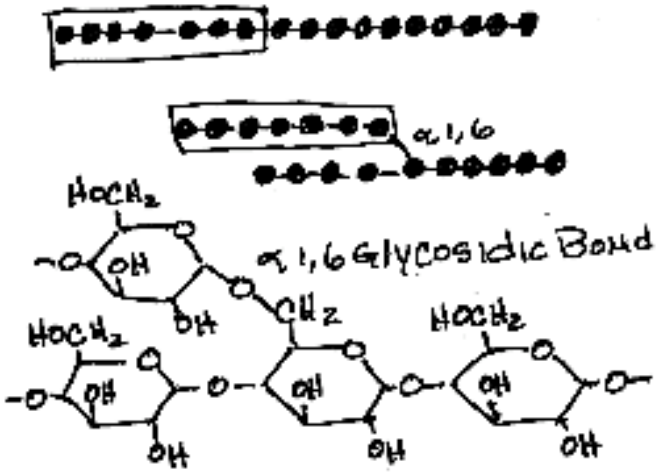
No 34: UDP-Glucose Pyrophosphorylase



No 35: Glycogen Synthase

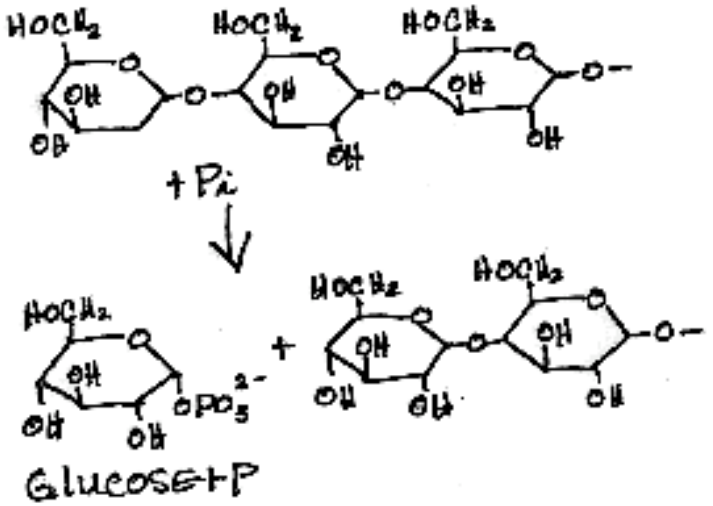


No 36: Branching Enzyme



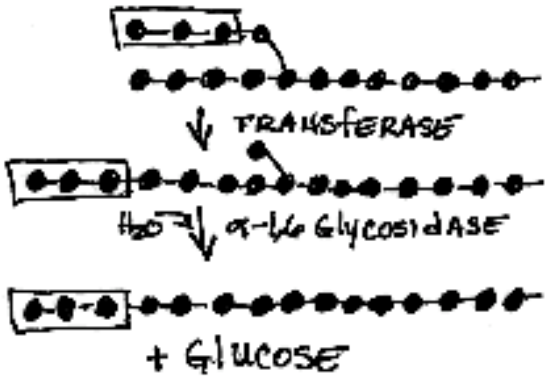
No 37: Glycogen Phosphorylase

$$(Glucose)_n + Pi \rightleftharpoons (Glucose)_{n-1} + Glucose\text{-}1\text{-}P$$



No 38: Debranching Enzyme

(Transferase & α 1,6 Glycosidase both in one protein)



No 39: = No 1 & No 33

$$Glucose + ATP \xrightarrow{\#1} Glucose\text{-}6\text{-}P + ADP$$

$$Glucose\text{-}6\text{-}P \underset{}{\overset{\#33}{\rightleftharpoons}} Glucose\text{-}1\text{-}P$$

#1 = Hexokinase or Glucokinase
#33 = phosphoglucomutase

IRREVERSIBLE AND REGULATED STEPS OF GLUCOSE METABOLISM

Trapping Glucose In Cells And Release Of Glucose To Blood

Phosphorylation of Glucose to yield Glucose-6-phosphate

The first step in glucose utilization is its phosphorylation, which prevents its transport out of cells due to the negative charge conferred by the phosphate group.

1. Hexokinase:	irreversible; allosterically inhibited by its product, glucose 6-phosphate; K_m = 0.1 mM
Glucokinase:	present in liver and pancreatic beta cells; irreversible; NOT inhibited by its product, glucose 6-phosphate; K_m = 10 mM; when blood glucose levels are low the enzyme level is reduced (transcription of the gene is reduced and the glucokinase protein is sequestered by the Glucokinase Regulatory Protein), thereby reducing the retention of glucose produced by glycogenolysis and gluconeogenesis in liver cells

Dephosphorylation of Glucose-6-phosphate to yield Glucose

The dephosphorylation of glucose allows it to be transported out of cells.

32. Glucose-6-phosphatase:	present in liver and kidney; located in the endoplasmic reticulum; irreversible; allows liver and kidney to supply glucose, recovered from stored glycogen or synthesized from 3-carbon precursors by gluconeogenesis, to other tissues during fasting; liver is the major tissue which supplies glucose to other tissues; during severe fasting the kidney also supplies an appreciable amount of glucose; gene transcription up regulated in response to increased cAMP (via CREB), down regulated by insulin.

Glycolysis and Gluconeogenesis

Phosphorylation of Fructose-6-phosphate to yield Fructose-1,6-bisphosphate

This is the committed step of glycolysis, and is highly regulated.

3. Phosphofructokinase 1:	irreversible; allosterically regulated: ATP increases the K_m for fructose 6-phosphate thereby slowing enzymatic activity and the rate of glycolysis; AMP reverses the inhibitory effect of ATP; inhibited by low pH; inhibited by high citrate concentration which enhances the inhibitory effect of ATP; stimulated by fructose-2,6-bisphosphate which lowers the K_m for fructose-6-phosphate and decreases inhibition by ATP thereby increasing the rate of glycolysis

Dephosphorylation of Fructose-1,6-bisphosphate to yield Fructose-6-phosphate

Note that the dephosphorylation of fructose-1,6-bisphosphate and the phosphorylation of fructose-6-phosphate are reciprocally regulated by AMP, fructose-2,6-bisphosphate and citrate. This prevents glycolysis and gluconeogenesis from occurring simultaneously.

31. Fructose-1,6 bisphosphatase:	irreversible; allosterically regulated: inhibited by AMP; inhibited by fructose-2,6 bisphosphate; inhibited by fructose-6 phosphate; stimulated by citrate

Phosphorylation of Fructose-6-phosphate to yield Fructose-2,6-bisphosphate and dephosphorylation of Fructose-2,6-bisphosphate to yield Fructose-6-phosphate by two different activities of a single protein, a bifunctional enzyme

Fructose-2,6-bisphosphate is synthesized by phosphofructokinase 2 in small amounts and acts as a "feed-forward" activator of phosphofructokinase 1. Fructose-6-phosphate stimulates the synthesis of fructose-2,6-bisphosphate, which then stimulates phosphofructokinase 1 to synthesize fructose-1,6-bisphosphate. The bifunctional enzyme is a single protein that has both phosphofructokinase 2 and fructose-2,6-bisphosphatase activities. Its activity is regulated by protein phosphorylation/dephosphorylation. Note below the reciprocal regulation of the two opposing enzymatic activities of this single protein.

3a. Phosphofructokinase 2:	stimulated by fructose-6-phosphate; Cyclic AMP-dependent protein kinase (protein kinase A) phosphorylates and inhibits the liver enzyme in response to either glucagon, which signals low blood glucose levels, or epinephrine; heart muscle isoform is stimulated by phosphorylation in response to epinephrine, skeletal muscle isoform is not phosphorylated.
3a. Fructose-2,6-bisphosphatase:	inhibited by fructose-6-phosphate; Cyclic AMP-dependent protein kinase (protein kinase A) phosphorylates and stimulates the liver enzyme in response to either glucagon, which signals low blood glucose levels, or epinephrine.

Production of Pyruvate from Phosphoenolpyruvate

This is the last step of glycolysis and is highly regulated to control the outflow of glycolytic products.

10. Pyruvate Kinase:	irreversible; ATP produced; allosterically regulated: inhibited by ATP; inhibited by alanine; "feed forward" stimulated by fructose-1,6-bisphosphate which signals a high level of glycolytic products are available; regulated by phosphorylation: cyclic AMP-dependent protein kinase (protein kinase A) phosphorylates and inactivates the liver (but not the muscle) enzyme in response to low blood glucose signalled by glucagon

Production of Phosphoenolpyruvate from Pyruvate

This is a two step reaction in which oxaloacetate is first produced from pyruvate and then phosphoenolpyruvate is produced from oxaloacetate. The two steps expend more energy than is harvested when pyruvate is produced from phosphoenolpyruvate.

29. Pyruvate carboxylase:	irreversible; oxaloacetate produced; ATP expended; located in mitochondria; allosterically regulated: activated by acetyl CoA, inhibited by ADP; the oxaloacetate produced is reduced (at the expense of NADH + H^+) to malate for transport from the mitochondria to the cytoplasm, where it is re-oxidized to oxaloacetate (at the expense of NAD^+); Note that the stimulation of oxaloacetate production during acetyl CoA excess could help speed up the TCA cycle by providing sufficient oxaloacetate to combine with the excess acetyl CoA, but if a surplus of NADH is available the oxaloacetate is directed towards gluconeogenesis as the TCA cycle slows.
30. Phosphoenolpyruvate carboxykinase:	inhibited by ADP; GTP expended: the phosphate of GTP is transferred to oxaloacetate and CO_2 is lost to generate phosphoenolpyruvate; transcription of the gene is regulated by the activity of cyclic AMP-dependent protein kinase (protein kinase A), and is responsive to blood glucose levels via the glucagon and insulin signaling pathways.

Acetyl CoA

Formation of Acetyl CoA from Pyruvate

Acetyl CoA is the link between glycolysis and the TCA cycle. Pyruvate, a 3-carbon structure, is converted to acetyl CoA with the loss of CO_2. The 2-carbon acetate subsequently enters the TCA cycle where it is completely oxidized to 2 CO_2.

12. Pyruvate dehydrogenase:	irreversible; occurs in mitochondria; inhibited competitively by acetyl CoA (note that acetyl CoA stimulates pyruvate carboxylase which directs pyruvate towards gluconeogenesis) and by NADH which are products of the reaction; regulated by a protein kinase that is NOT the cyclic AMP-dependent protein kinase: phosphorylation of pyruvate dehydrogenase, which is stimulated by high $NADH/NAD^+$, acetyl CoA/CoA or ATP/ADP inhibits enzymatic activity; pyruvate activates by inhibiting the protein kinase; insulin stimulates by activating a phosphatase that removes the protein phosphate; Ca^{2+}, released from the sarcoplasmic reticulum during muscle contraction, activates the phosphatase thereby speeding up the flow of pyruvate into the TCA cycle for ATP production to power muscle contraction

RECIPROCAL REGULATION OF GLYCOLYSIS AND GLUCONEOGENESIS				
GLYCOLYSIS			GLUCONEOGENESIS	
Enzyme	Regulation		Enzyme	Regulation
Hexokinase	product inhibited		Glucose-6-phosphatase	only in liver and kidney
Glucokinase	not product inhibited only in liver			
Phosphofructokinase I	F-2,6-BP	↑	Fructose-1,6-bisphosphatase	F-2,6-BP ↓
	AMP	↑		AMP ↓
	ATP	↓		citrate ↑
	citrate	↓		F-6-P ↓
	low pH	↓		
Pyruvate kinase	F-1,6-BP	↑	Pyruvate carboxylase	acetyl CoA ↑
	ATP	↓		ADP ↓
	alanine	↓	Phosphoenol pyruvate carboxykinase	ADP ↓
	glucagon	↓		
Pyruvate dehydrogenase	pyruvate	↑	There is no opposing enzymatic step. Acetyl CoA cannot be used to generate pyruvate.	
	insulin	↑		
	acetyl CoA	↓		
	ATP	↓		
	NADH	↓		

The TCA Cycle

The final step in the oxidation of fuel molecules. Acetate generated from glucose (and other fuel molecules) as acetyl CoA is ultimately oxidized to 2 CO_2 with the generation of 1 GTP and 4 reducing equivalents (3 NADH and 1 $FADH_2$) from each acetate molecule that enters the cycle. These reducing equivalents are used to generate ATP via oxidative phosphorylation, where oxygen is the ultimate electron acceptor.

Formation of alpha-Ketoglutarate from Isocitrate

The first of four oxidation reactions of the TCA cycle and the first decarboxylation

15. Isocitrate dehydrogenase: irreversible; NADH and CO_2 produced (oxidative decarboxylation); actually a two step reaction in which oxidation of isocitrate to oxalosuccinate occurs first, followed by decarboxylation; ADP enhances the cooperative binding of NAD^+ and isocitrate to the enzyme; ATP inhibits; NADH inhibits by displacing NAD^+ from the enzyme. Ca^{2+}, released from skeletal muscle sarcoplasmic reticulum, speeds up the enzyme to supply increaased energy for muscle contraction.

Formation of Succinyl CoA from alpha-Ketoglutarate and CoA

The second of four oxidation reactions of the TCA cycle and the second decarboxylation

16. α-ketoglutarate~dehydrogenase:	Irreversible; NADH and CO_2 produced (oxidative decarboxylation); inhibited by NADH and succinyl CoA; the oxidation allows the formation of the energy-rich thioester. Ca^{2+}, released from skeletal muscle sarcoplasmic reticulum speeds up the enzyme to supply increaased energy for muscle contraction.

Glycogenesis and Glycogenolysis

Glycogenesis and glycogenolysis are reciprocally regulated.

The two opposing, regulated enzymes are glycogen synthase (glycogenesis) and glycogen phosphorylase (glycogenolysis). Each is regulated by phosphorylation/dephosphorylation and by binding of other molecules. Phosphorylation of glycogen synthase switches it to the inactive, or "*b* " form. Phosphorylation of glycogen phosphorylase switches it to the active, or "*a* " form. The "*b* " forms of each enzyme can become active by binding small molecules. The differential response of the liver and muscle isoforms to different small molecules (glucose, AMP, ATP -see below) is in keeping with the different functions of liver and muscle in glucose metabolism. Muscle uses glucose as a primary fuel, on site, while the liver does not use glucose as a primary fuel, but rather acts to maintain blood glucose homeostasis. Glycogen synthase is phosphorylated directly by cyclic AMP-dependent protein kinase (protein kinase A) in response to glucagon or epinephrine. Glycogen phosphorylase is phosphorylated by phosphorylase kinase, which becomes active when phosphorylated by cyclic AMP-dependent protein kinase (protein kinase A). Protein phosphatase 1, which removes protein phosphates, reverses the effect of phosphorylation. Protein phosphatase 1 is regulated by two mechanisms: (1) when phosphorylated by cyclic AMP-dependent protein kinase (protein kinase A), its G (for glycogen binding) subunit is prevented from binding to glycogen particles, (2) when phosphorylated by cyclic AMP-dependent protein kinase (protein kinase A), inhibitor 1, a small protein, inhibits protein phosphatase 1. Insulin activates protein phosphatase 1.

A reciprocal relationship, effected by protein phosphatase 1, exists between the activities of glycogen phosphorylase (glycogen breakdown) and glycogen synthase (glycogen synthesis). Protein phosphatase 1 binds phosphorylase " *a* " when glucose is low, but does not dephosphorylate phosphorylase *a*. When glucose levels rise, glucose binds to phosphorylase *a* and causes a conformational change in its structure. This allows protein phosphatase 1 to dephosphorylate phosphorylase *a*, converting it to phosphorylase *b*, which is unable to bind protein phosphatase 1. Protein phosphatase 1 is thereby freed to dephosphorylate, and activate, glycogen synthase.

35. Glycogen synthase:	cyclic AMP-dependent protein kinase (protein kinase A) phosphorylates and inactivates (switches it to the "*b* " form), high level of glucose-6-phosphate overcomes the inhibition; insulin activates by activating protein phosphatase 1, which removes inhibiting phosphates from the synthase, thereby switching it to the active, or "*a* " form; glucose activates by causing the release of protein phosphatase 1 from phosphorylase so it becomes free to dephosphorylate glycogen synthase; epinephrine, acting through the cyclic AMP-dependent protein kinase pathway inactivates by causing phosphorylation; epinephrine, acting through a different pathway (not discussed here) and muscle contraction cause in an increase in intracellular Ca^{2+}, which subsequently activates different protein kinases to phosphorylate and inactivate glucogen synthase

37. Glycogen phosphorylase:	cyclic AMP-dependent protein kinase phosphorylates phosphorylase kinase, thereby activating it to phosphorylate glycogen phosphorylase, which is thereby activated (phosphorylase *a*); protein phosphatase 1 dephosphorylates thereby inactivating it (phosphorylase *b*); the muscle "*b* " isoform is activated by AMP, ATP reverses the effect of AMP by competing with it for binding to the enzyme; the muscle "*b* " isoform is inactivated by glucose-6-phosphate; AMP has no effect on the liver isoform of phosphorylase; the liver isoform of phosphorylase is deactivated by the binding of glucose, which causes a conformational change and exposes the protein phosphate to the dephosphorylating activity of protein phosphatase 1; epinephrine, acting through the cyclic AMP-dependent protein kinase pathway activates by causing phosphorylation or phosphorylase kinase; epinephrine, acting through a different pathway (not discussed here) and muscle contraction cause in an increase in intracellular Ca^{2+}, which subsequently activates a different protein kinase to phosphorylate and activate glycogen phosphorylase