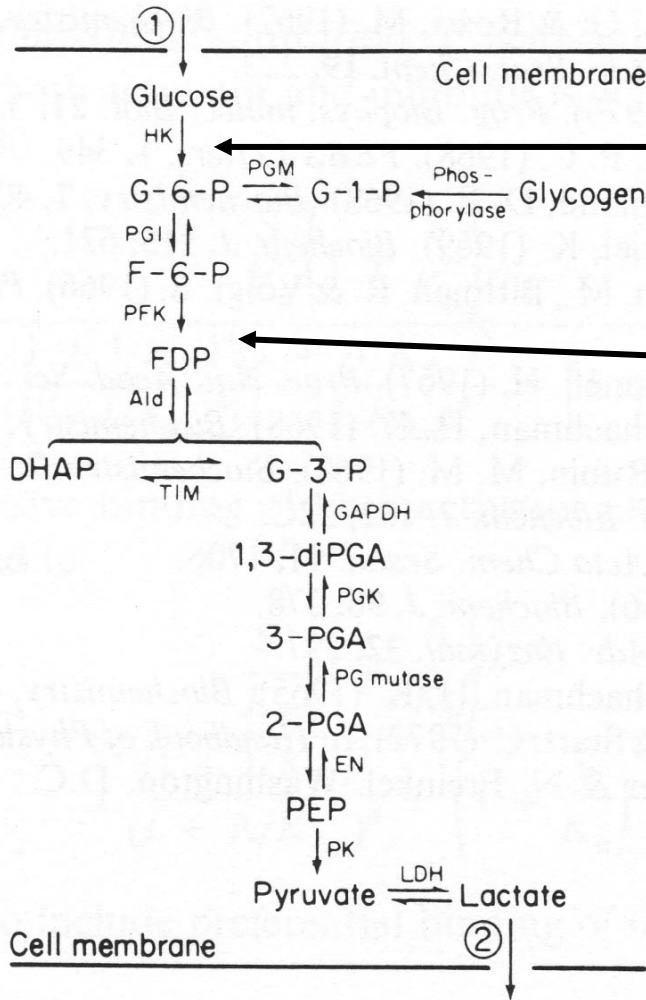


ANSC 619
PHYSIOLOGICAL CHEMISTRY OF LIVESTOCK SPECIES
Carbohydrate Metabolism

I. Glycolysis

A. Pathway



Regulation of glycolysis

Hexokinase:

Activated by glucose.

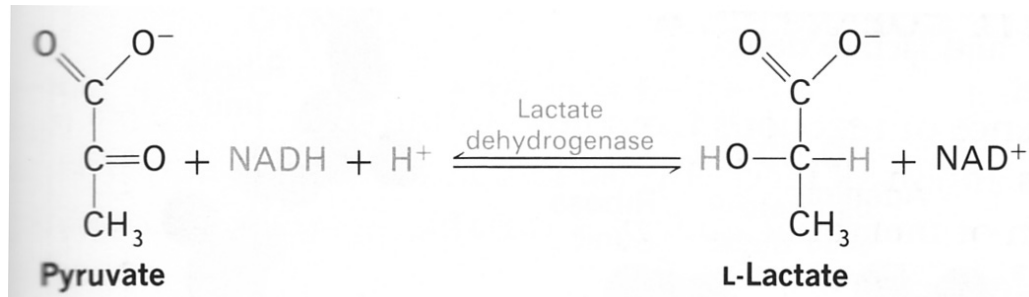
Inhibited by G6P.

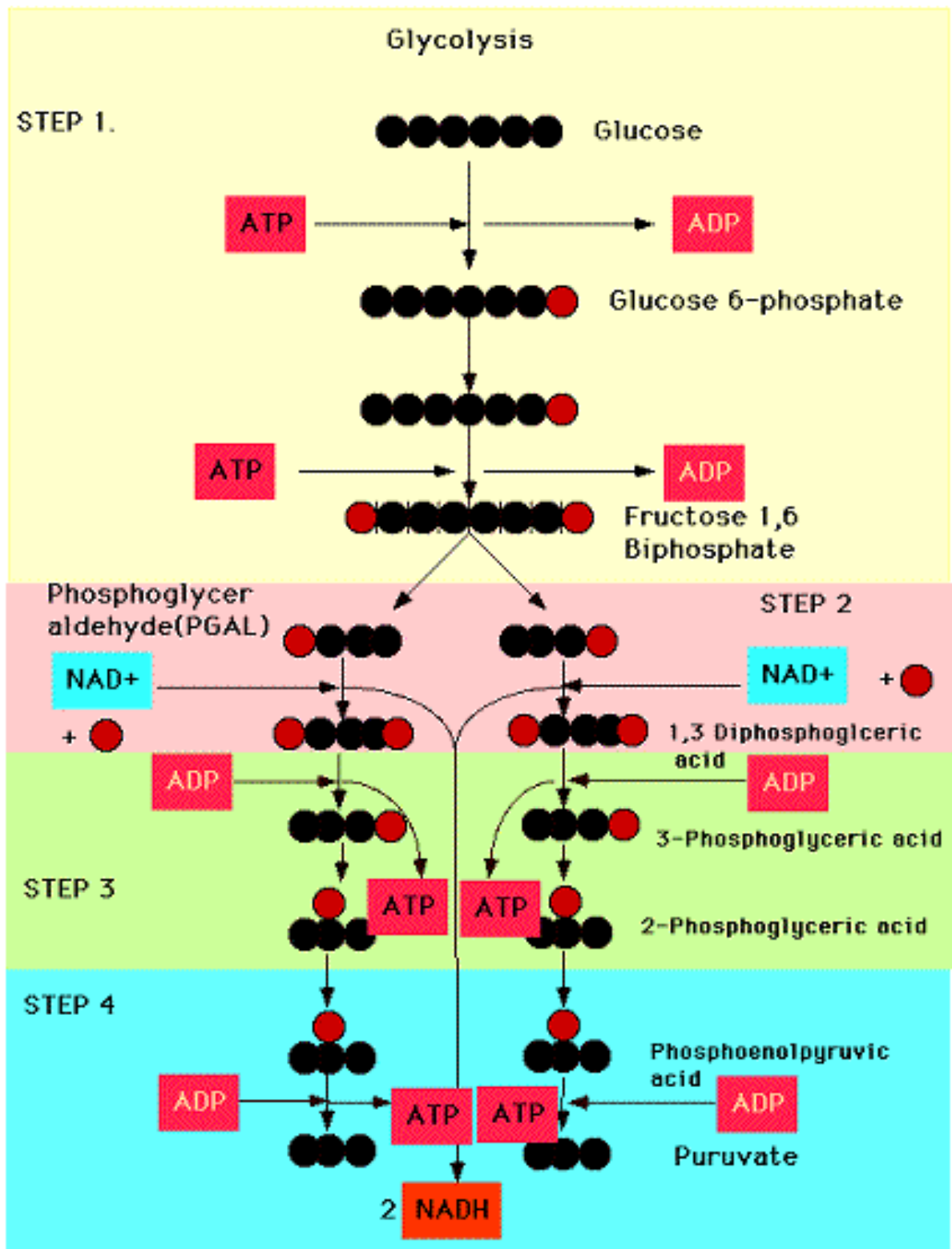
6-Phosphofructokinase:

Inhibited by ATP, especially in the presence of citrate.

Activated by AMP.

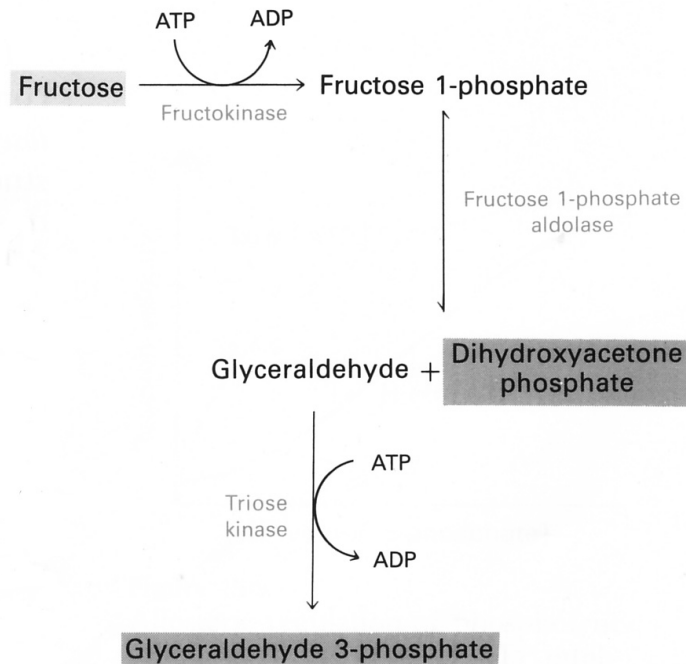
B. Pyruvate and lactate





C. Entry of fructose into glycolysis

1. Fructose – Phosphorylated by fructokinase to fructose-1-phosphate, then split to DHAP and glyceraldehyde.



II. Krebs (tricarboxylic acid or citric acid) cycle

A. Conversion of pyruvate to acetyl CoA

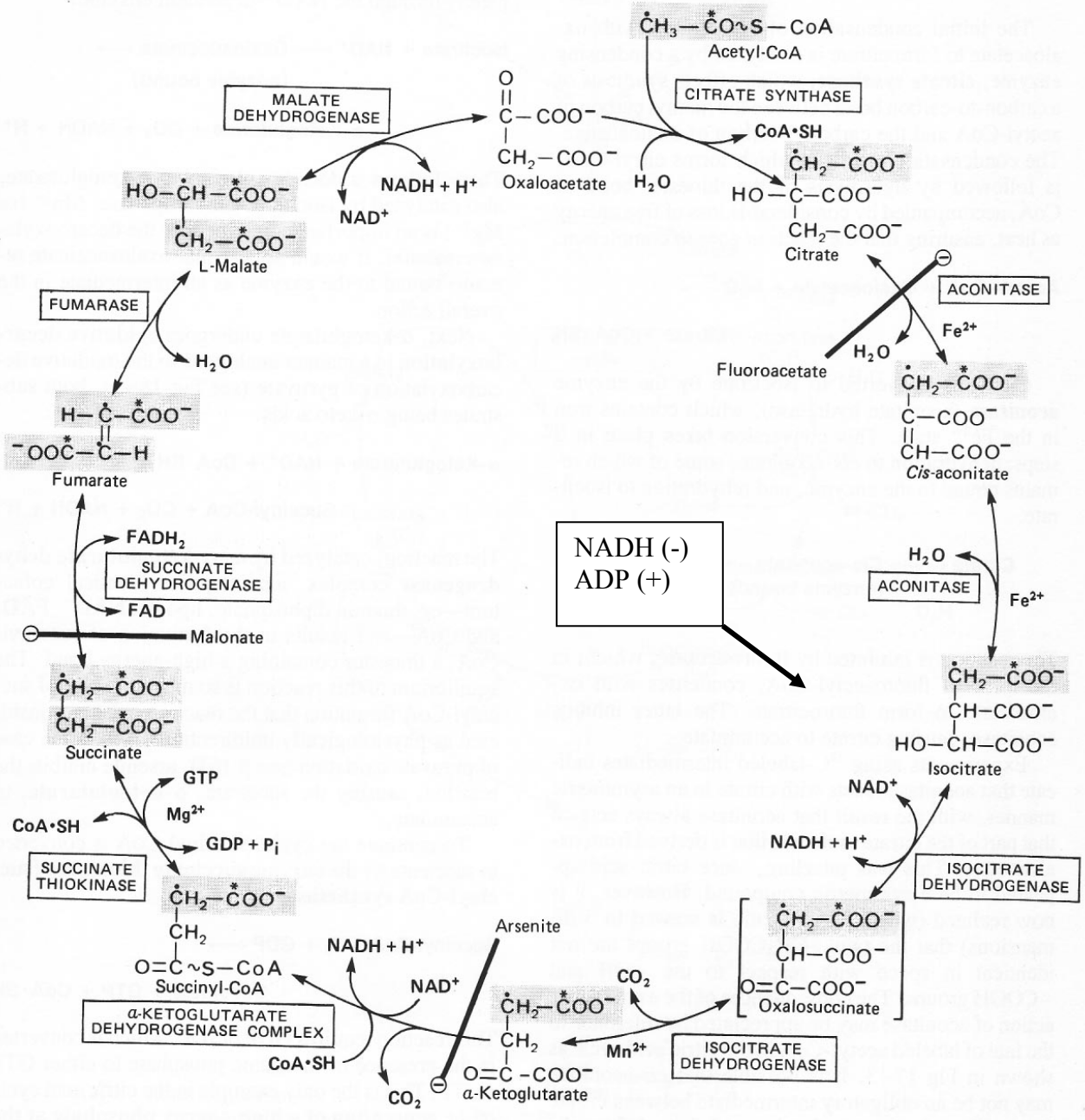
1. Pyruvate is decarboxylated by pyruvate dehydrogenase at the inner mitochondrial membrane.
2. Coenzyme A is attached by a thioester bond to acetate to form acetyl-CoA

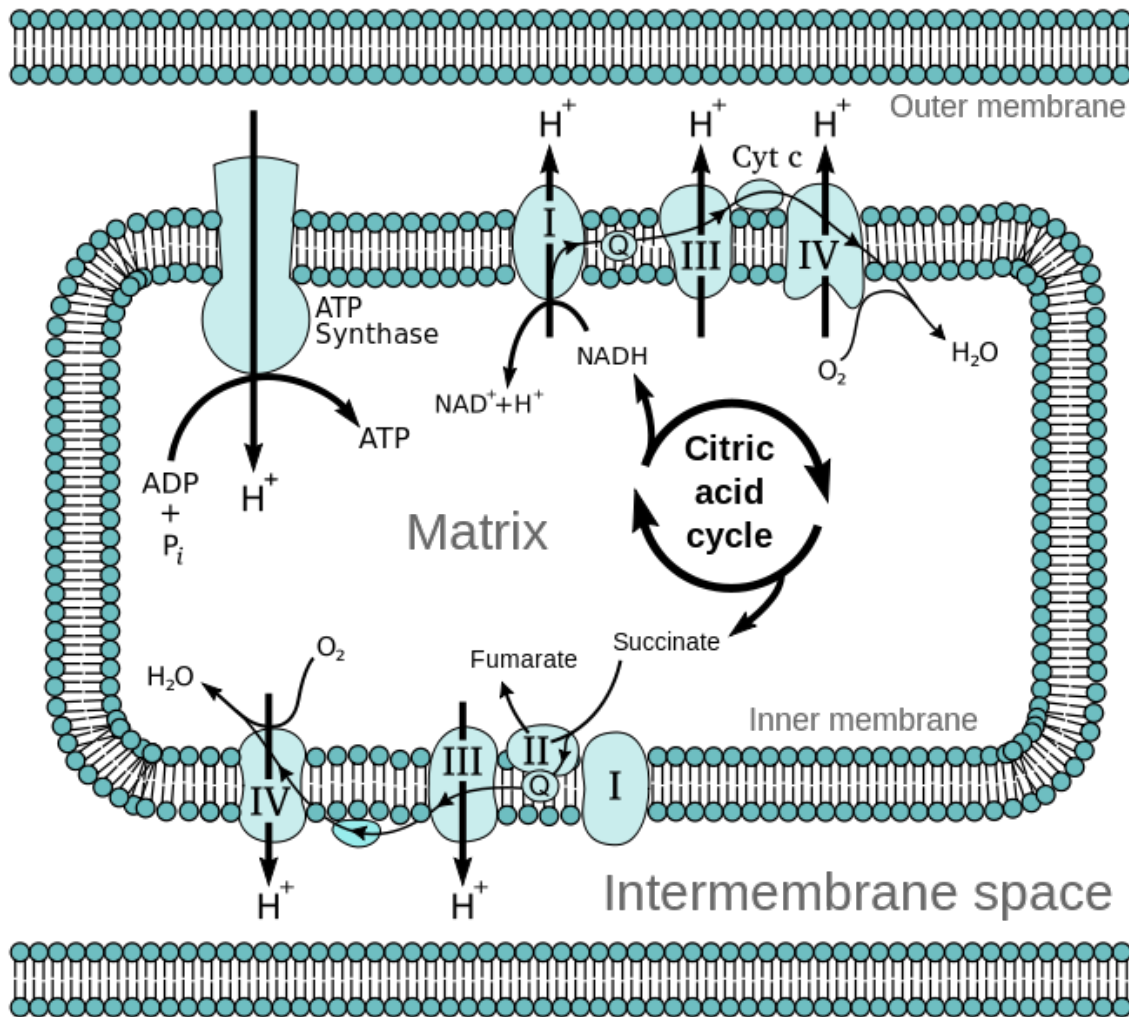
B. Conversion of pyruvate to oxaloacetate

1. Pyruvate crosses the inner mitochondrial membrane.
2. Pyruvate is carboxylated to oxaloacetate in the mitochondrial matrix.

C. The cycle

1. Oxaloacetate condenses with acetyl-CoA to initiate the TCA cycle.
2. A net of two carbons is lost during one complete cycle.
3. Primary regulation is at isocitrate dehydrogenase.





III. Energy production by the electron transport system

A. NADH is oxidized by the electron transport system beginning with the first reductase.

1. Protons are pumped out of the mitochondrial matrix at three sequential reductases.
2. 3 ATP are synthesized by ATP synthase as protons (H^+) flow along their concentration gradient back into the mitochondrial matrix.

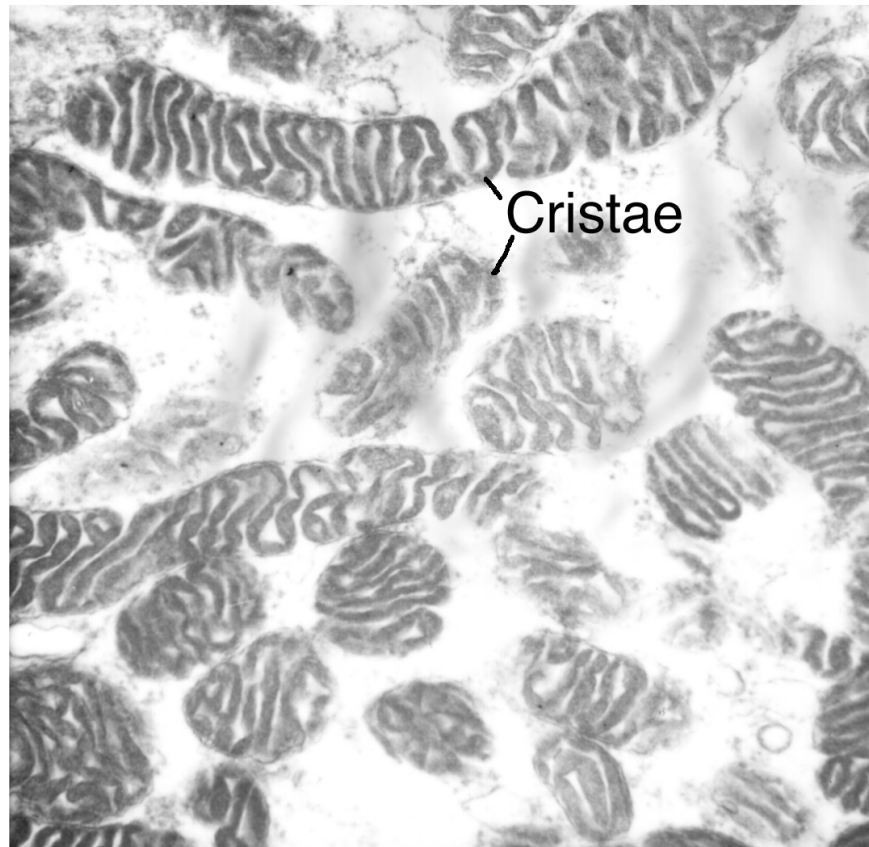
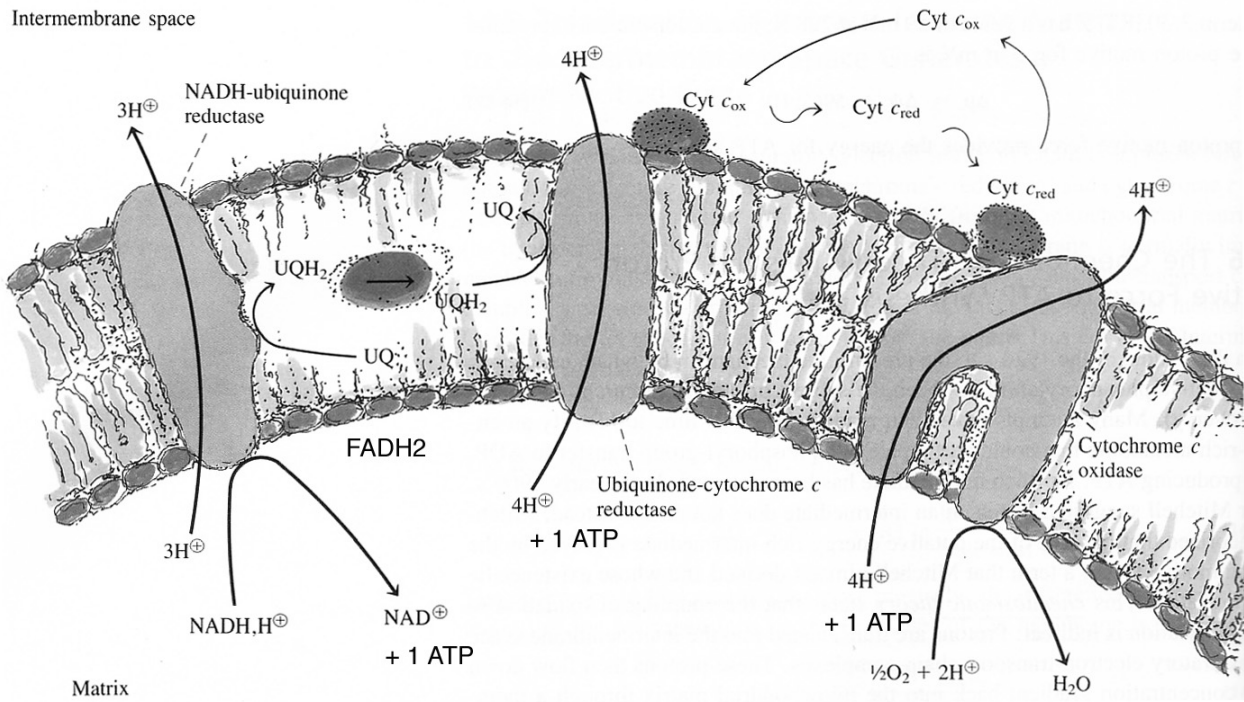
B. $FADH_2$ enters the electron transport system at the second reductase.

1. Protons are pumped out of the mitochondrial matrix at two of the reductases.
 - a. The first reductase includes succinate dehydrogenase.
 - b. This reductase uses FAD to transport electrons and protons.
2. 2 ATP are synthesized by ATP synthase.

C. Oxygen is the terminal electron acceptor.

1. Electrons and protons are transferred to $\frac{1}{2} O_2$.

2. Metabolic water is produced.



IV. Gluconeogenesis

A. Essential enzymes

1. Pyruvate carboxylase (converts pyruvate to oxaloacetate)
2. Phosphoenolpyruvate carboxykinase (PEPCK) (converts oxaloacetate to PEP)
3. Fructose 1,6-bis-phosphatase (converts fructose 1,6-bisphosphate to F-6-P).
4. Glucose-6-phosphatase (converts G-6-P to free glucose)

B. Overall pathway

Pyruvate → oxaloacetate → PEP → → Fructose 1,6-diphosphate → F-6-P → G-6-P → **Glucose**

C. Organs responsible for gluconeogenesis

- A. Liver – produces glucose for the rest of the body
- B. Kidney cortex – produces glucose for its own use

V. Fermentation

A. Products of fermentation

1. All carbohydrates are converted eventually to glucose
2. Glucose is converted to pyruvate which can be reduced to lactate.

B. Entry of pyruvate into the TCA cycle in bacteria and other ruminal microorganisms

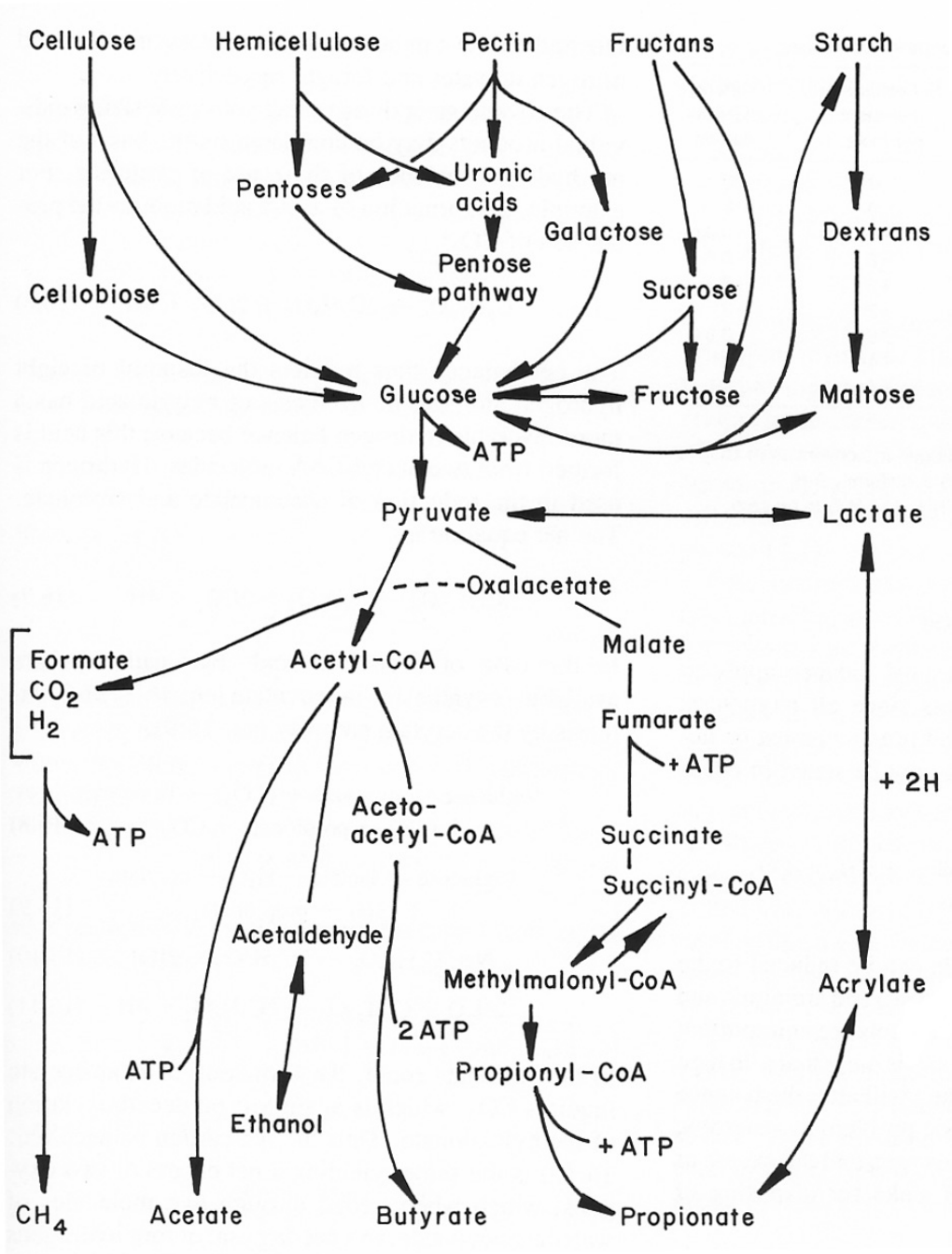
1. As in mitochondria, pyruvate is decarboxylated to acetyl-CoA or carboxylated to oxaloacetate.
2. The “cycle” then is reversed: OAA is reduced to malate, which is isomerized to fumarate, then to succinate, and finally to succinyl-CoA.

C. Formation of volatile fatty acids

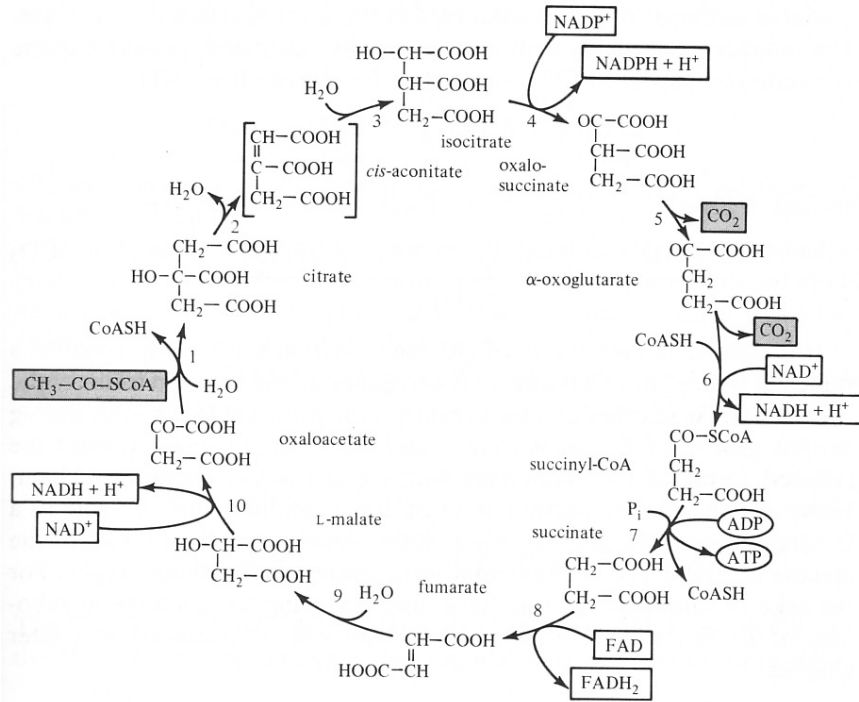
1. Succinyl-CoA is converted to the branched-chain methylmalonyl-CoA.
2. Methylmalonyl-CoA is demethylated to propionyl-CoA.
3. Propionyl-CoA loses its CoASH group (hydrolysis of the thioester bond) to produce propionate (3 carbons).
 - a. In eukaryotic liver cells (especially in ruminants), propionate enters the TCA cycle by the reverse of this pathway.
 - b. Propionate is a critical source of carbon for gluconeogenesis in ruminants.
4. Acetyl-CoA can condense with a second acetyl-CoA to produce acetoacetyl-CoA (4 carbons), which then can be converted to butyrate.
5. Acetyl-CoA can be converted to acetate (2 carbons).

6. Oxaloacetate can be decarboxylated to formate, which then serves as the substrate for methane production (1 carbon). (Methane also can be produced from other VFAs.)

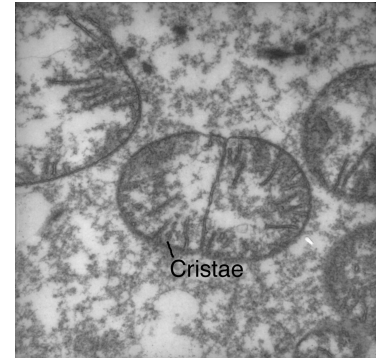
The production of all VFAs results in the net production of ATP for ruminal microorganisms.



B. TCA cycle in bacteria

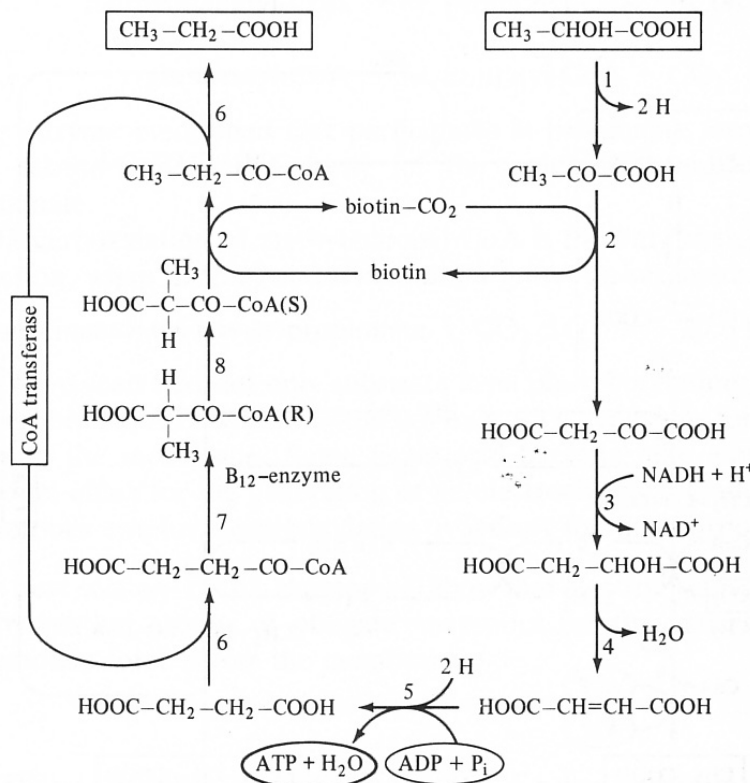


The TCA cycle is identical in bacteria and mitochondria of eukaryotes.



Eukaryotic mitochondrion

C. Propionate formation in bacteria



sum: lactate + NADH + H⁺ + ADP + P_i → propionate + NAD⁺ + ATP + 2H₂O

Propionate formation is a reversal of the entry of propionate into the TCA cycle.

The primary substrate for propionate is lactate, which is converted to pyruvate → OAA → malate → fumarate → succinate → succinyl-CoA → methylmalonyl-CoA → propionyl-CoA → propionate.

(To make glucose, bovine liver mitochondria partially reverse the pathway: Propionate → OAA Then: OAA → PEP → glucose

VI. Glycogen metabolism

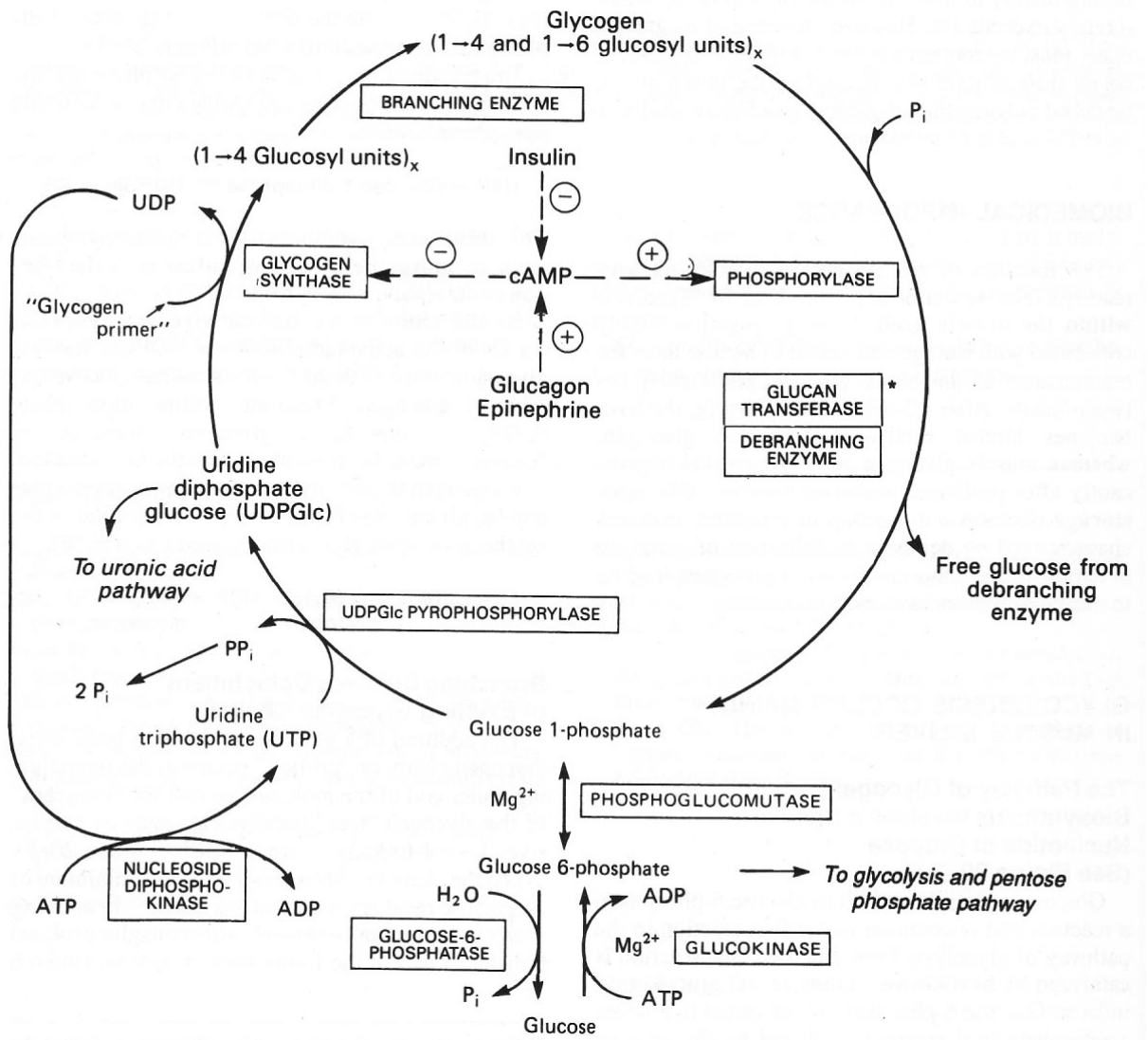
A. Liver

1. Contains up to 6% glycogen.
2. Provides glucose for systemic metabolism.

B. Muscle

1. Rarely exceeds 1% (very consistent).
2. Because of muscle mass, muscle contains three to four times as much glycogen as liver.

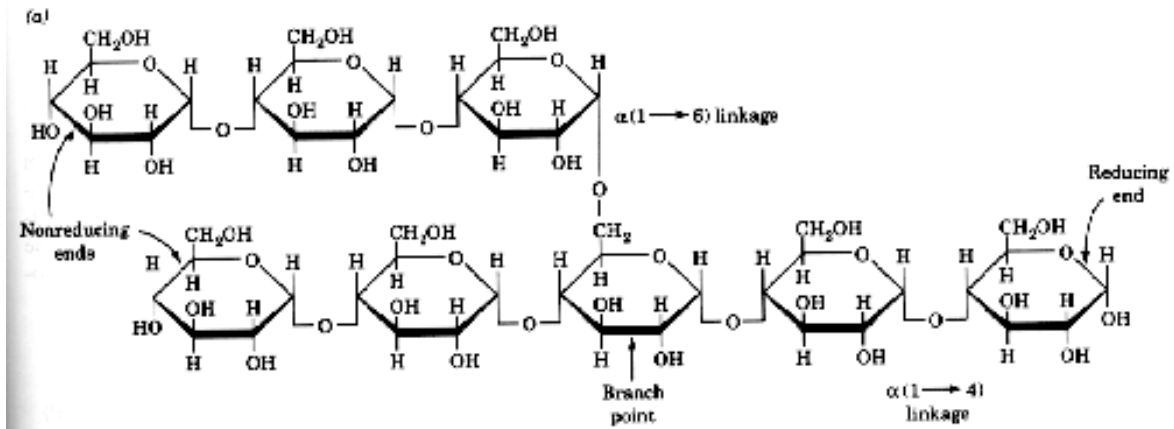
C. Overview of glycogen turnover



D. Glycogen branching

1. Structure of glycogen

- Backbone consists of α -1,4 glycosidic linkages.
- Branchpoints consist of α -1,6 glycosidic linkages.



2. Mechanism of branching

- 11 α -1,4 glycoside residues are added to a chain.
- The terminal six residues are transferred to an adjacent chain in a α -1,6 glycosidic linkage.

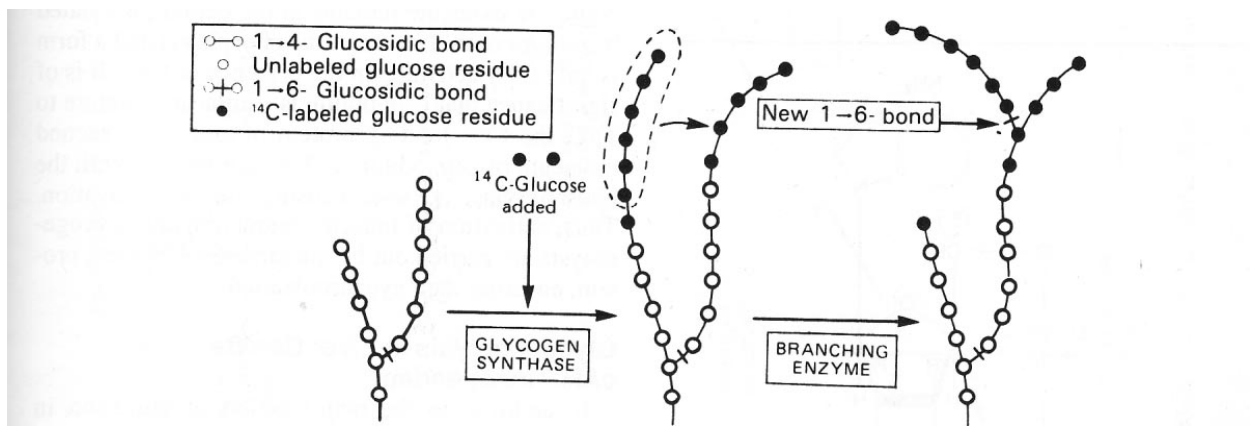
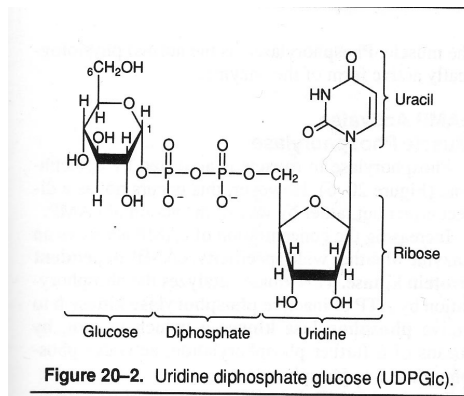
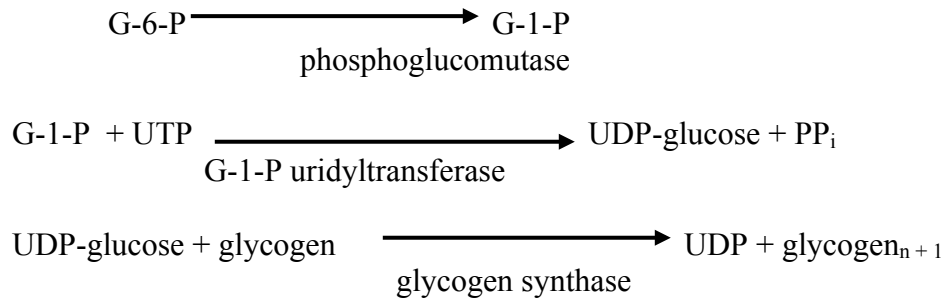


Figure 20-3. The biosynthesis of glycogen. The mechanism of branching as revealed by adding ¹⁴C-labeled glucose to the diet in the living animal and examining the liver glycogen at further intervals.

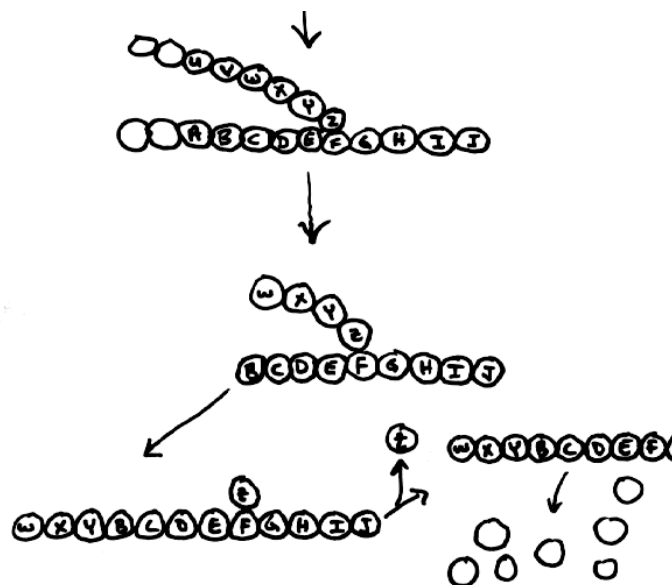
Glycogen synthesis



VII. Glycogen degradation

A. Glycogen phosphorylase adds phosphate groups to the 1-carbon of glucosyl residues of glycogen, producing G1P.

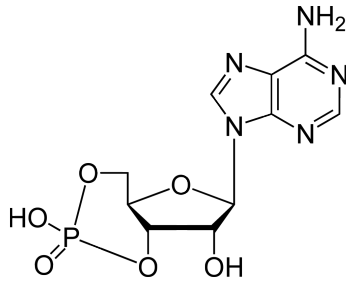
B. This reaction also produces free glucose at branch points.



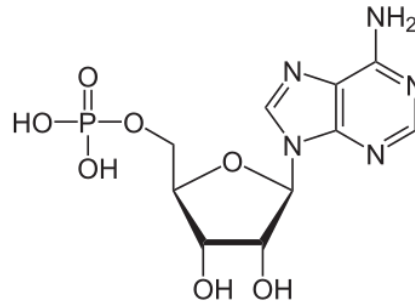
VIII. Regulation of glycogen synthesis

A. Epinephrine or glucagon binds to the cell receptor (β -receptor for epinephrine)

1. Hormone binding activates a G-regulatory protein complex.
2. Activated G-regulatory protein activates adenylate cyclase.
3. ATP is converted to cyclic AMP (cAMP).

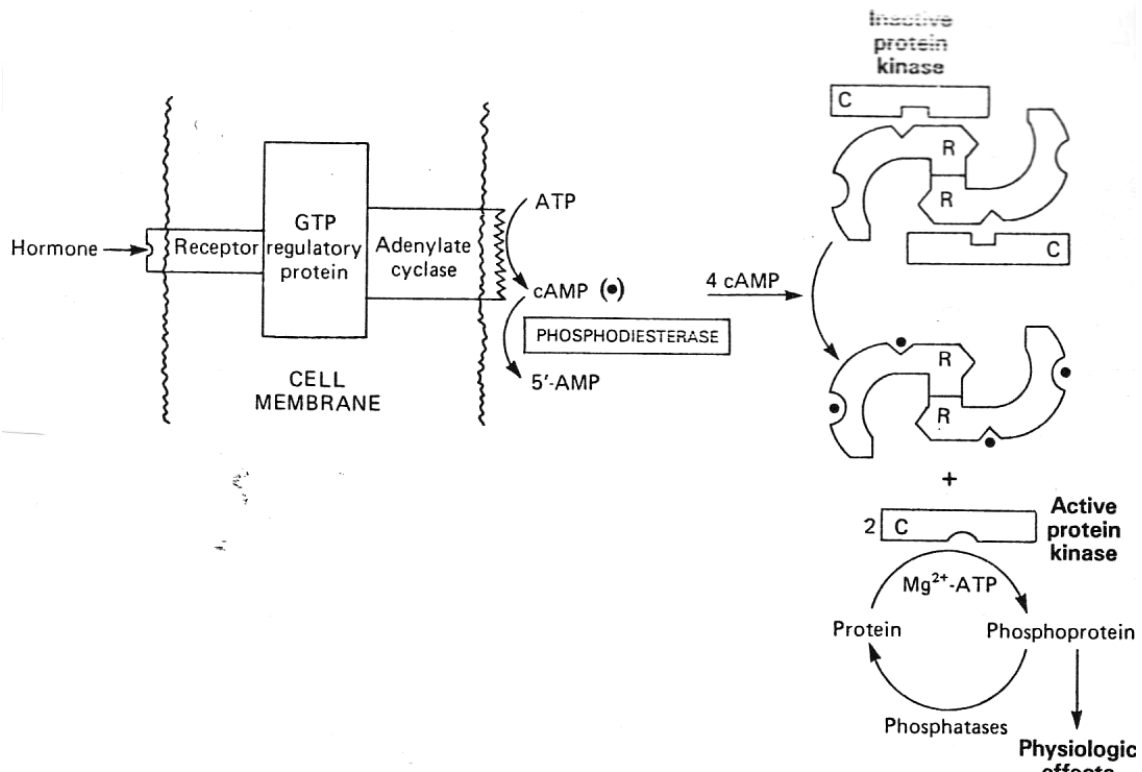


Cyclic AMP (cAMP)



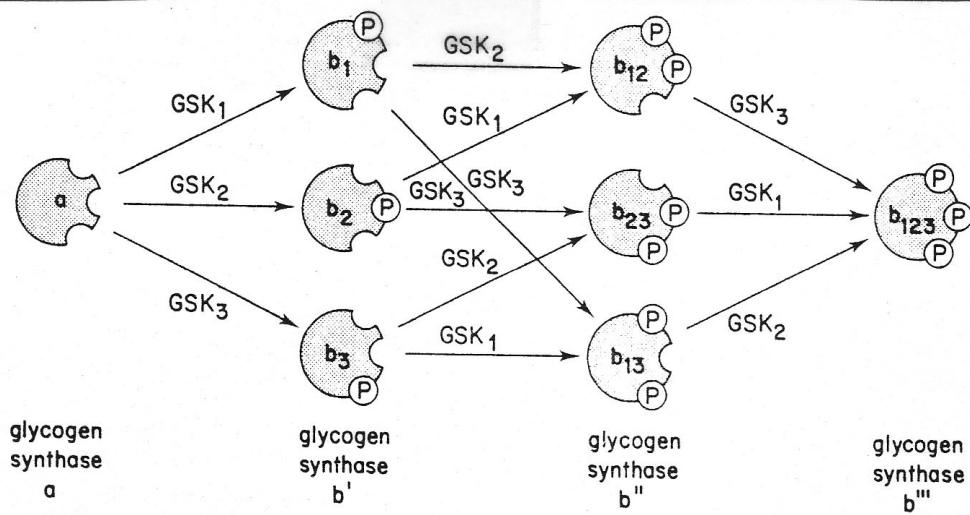
5'AMP

4. cAMP binds with regulatory subunits of protein kinase and frees catalytic subunits.
 - a. Active catalytic subunits of protein kinase phosphorylate enzymes at serine residues.
 - b. Phosphorylation inactivates synthetic enzymes and activates degradative enzymes.
 - c. Conversion of cAMP to AMP returns cells to their basal state.



B. Phosphorylation of glycogen synthase via epinephrine

1. GSK₁ (cAMP-dependent protein kinase) phosphorylates serine residues.
2. GSK₂ (phosphorylase kinase) phosphorylates serine residues.
3. GSK₃ (glycogen synthase-specific kinase) phosphorylates serine residues.



C. Action of insulin

1. Stimulates phosphatases, which activate glycogen synthase.
2. Increases glucose uptake, which provides G-6-P, which provides substrate for glycogen synthesis.

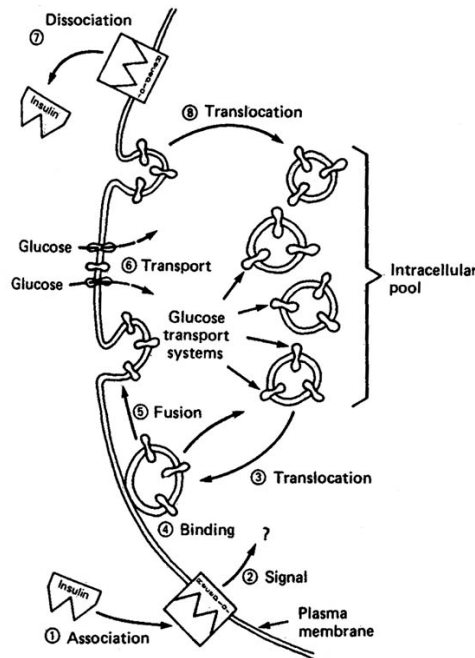


Figure 51-9. Translocation of glucose transporters by insulin. (Reproduced, with permission, from Karnieli E et al: Insulin-stimulated translocation of glucose transport systems in the isolated rat adipose cell. *J Biol Chem* 1981;**256**:4772. Courtesy of S Cushman.)

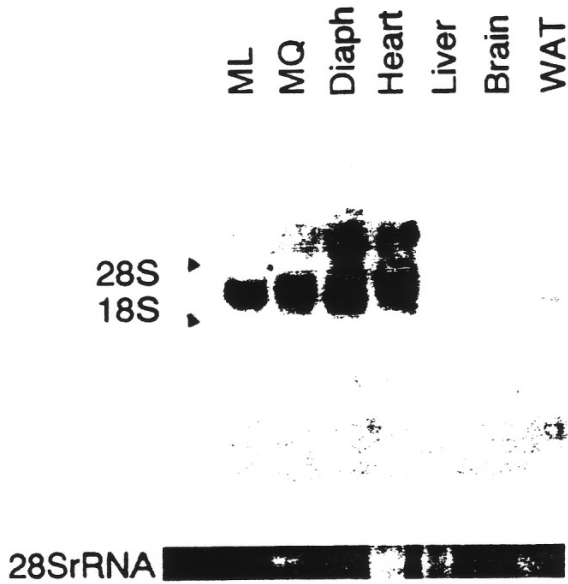
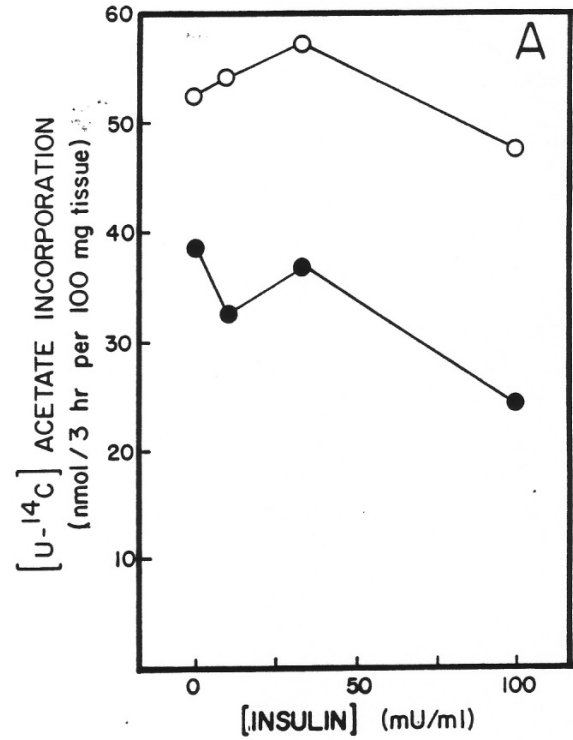
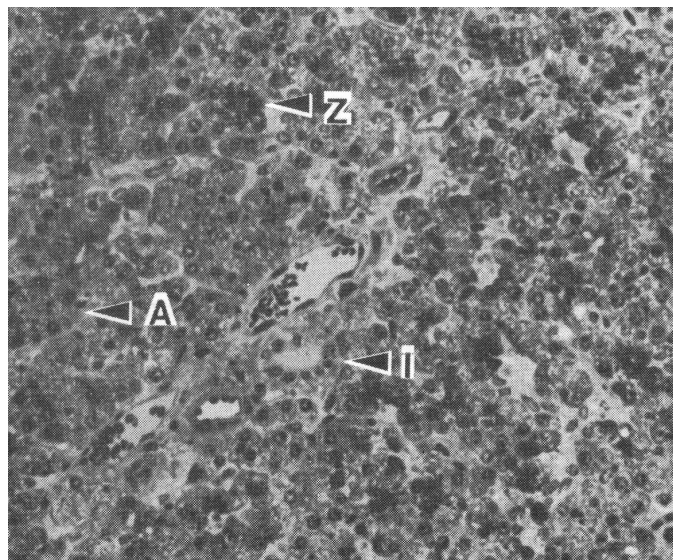


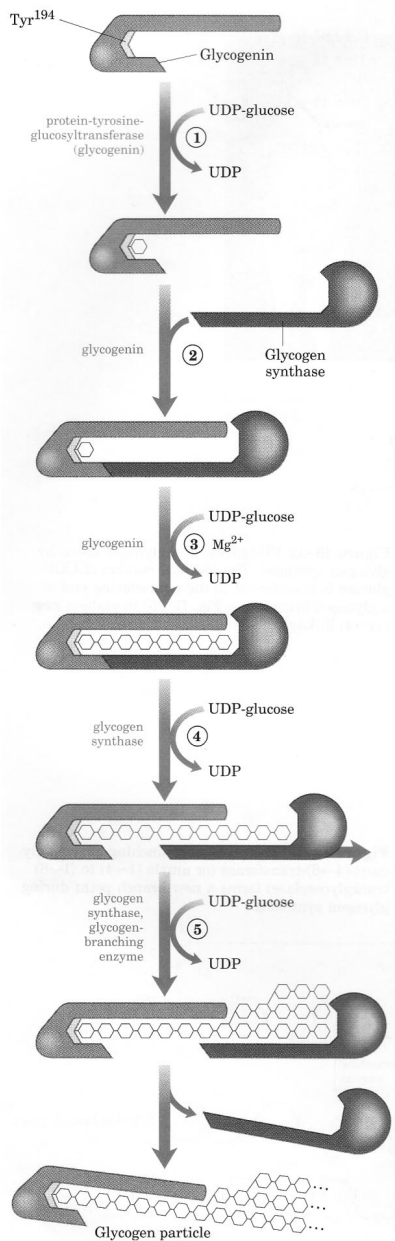
Figure 4. Tissue distribution of bovine GLUT4 mRNA. Thirty micrograms of total RNA was used for Northern blot analysis. ML: longissimus muscle, MQ: Quadriceps muscle, Diaph: diaphragm, WAT: perirenal white adipose tissue. The 28S rRNA band stained by ethidium bromide was also shown.



3. Insulin and metabolism in ruminants

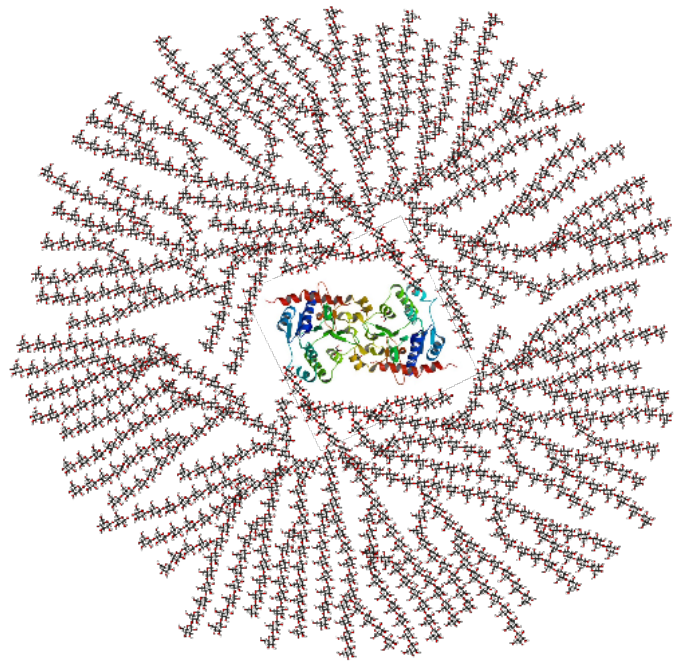
- a. Very few insulin receptors.
- b. Very low levels of glucose transporters and GLUT4 mRNA in adipose tissue and muscle, although the diaphragm and heart have high GLUT4 mRNA levels.
- c. Insulin has a limited ability to stimulate glucose uptake in bovine adipose tissue and skeletal muscle.





D. Role of glycogenin

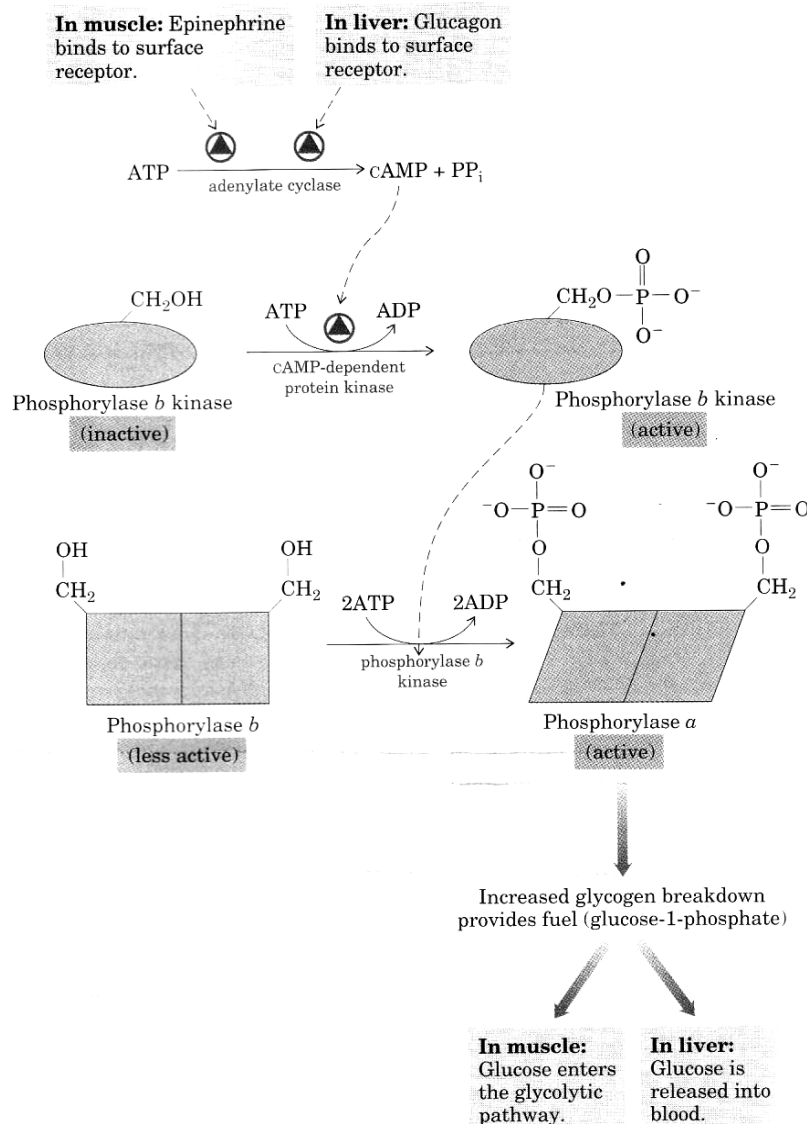
1. Binds glucose residues
2. Serves as primer for glycogen synthesis.
3. Catalyzes synthesis of initial glycogen polymer: 8 residues are condensed, after which glycogen synthase extends the molecule.
4. Forms the core of the β -particle (55,000 glucose residues).
5. Cross-sectional view of glycogen



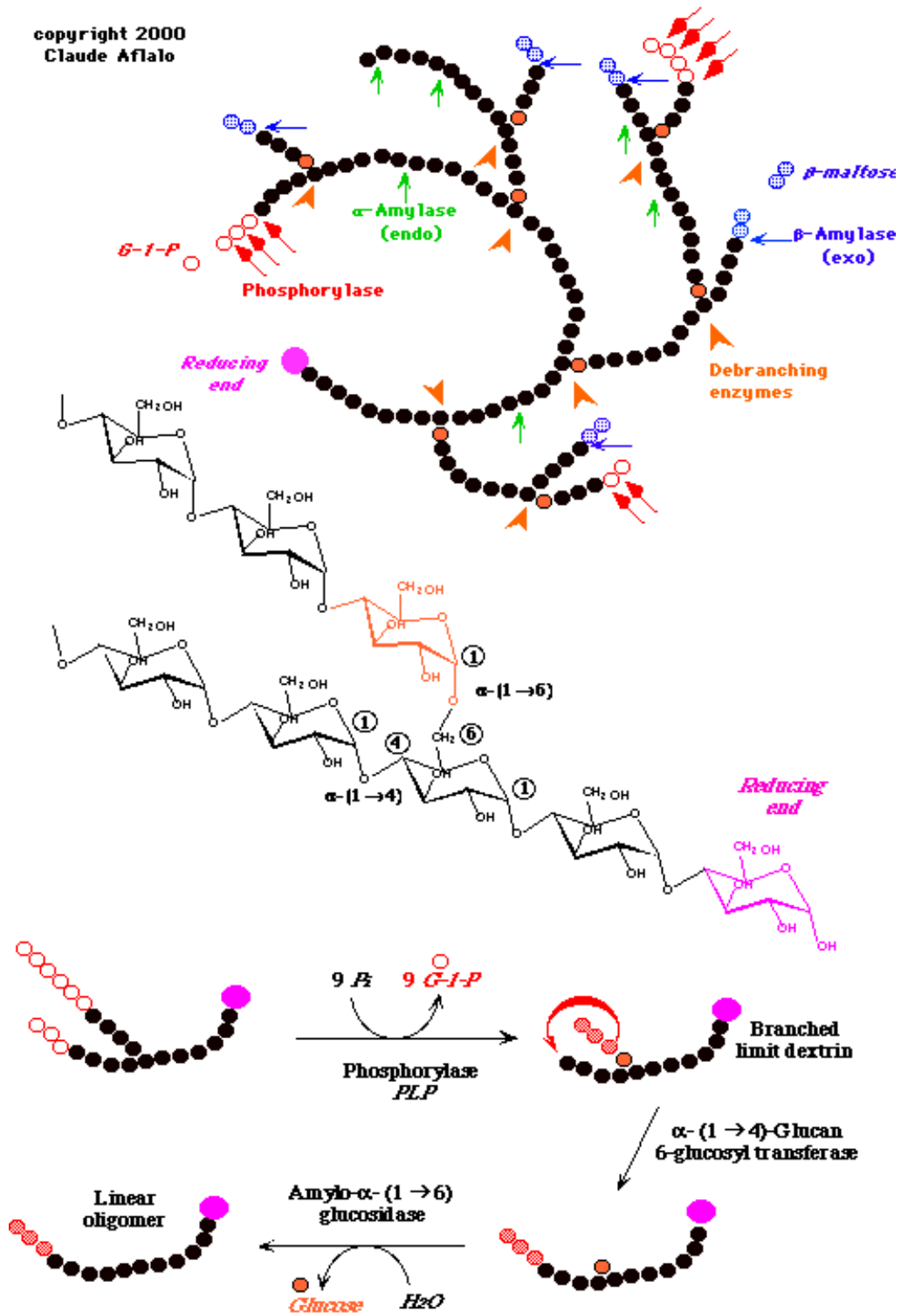
IX. Regulation of glycogen degradation

- A. Catalytic subunits (**protein kinase**) phosphorylate **phosphorylase kinase**.
- B. Phosphorylase kinase phosphorylates **glycogen phosphorylase**.
- C. Glycogen phosphorylase adds phosphate groups to the 1-carbon of glucosyl residues of glycogen, producing G1P.
 1. Phosphorylase_b (inactive phosphorylase) activity is modulated by:

- a. P_i and AMP, which activate phosphorylase_b
- b. ATP and G6P, which inhibit phosphorylase_b
- 2. Phosphorylase_a (active phosphorylase) activity is modulated by glucose, which inhibits its activity.
- D. Phosphorylase_a also produces free glucose at branch points.
- E. Phosphorylase kinase activity is regulated by Ca^{++} .
 - 1. Concentration of Ca^{++} required for activation of phosphorylase kinase is lower for the phosphorylated (active) form.
 - 2. Concentration required for activation of phosphorylase kinase is that which yields half-maximal stimulation of myosin ATPase (muscle contraction).

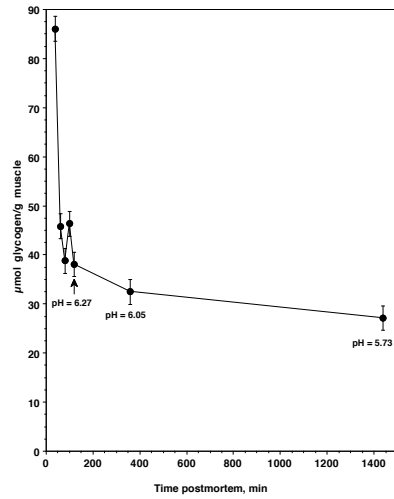


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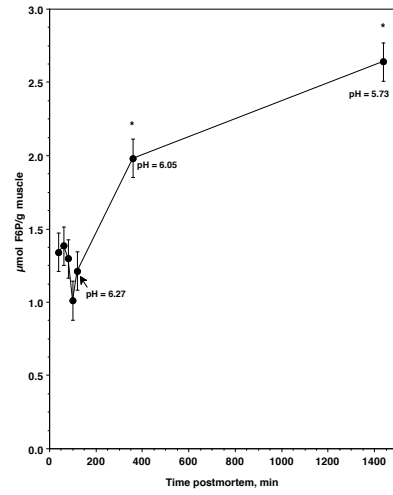


Postmortem metabolism in bovine muscle

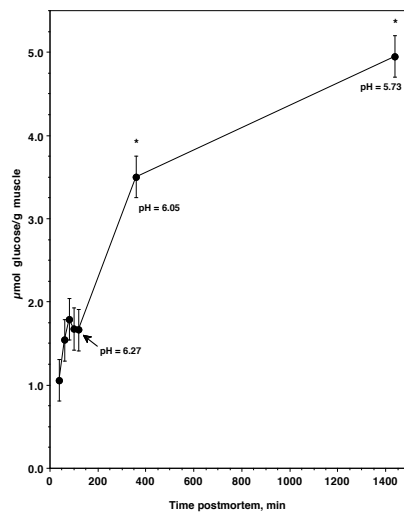
A. Muscle glycogen declined to about one-third of initial values.



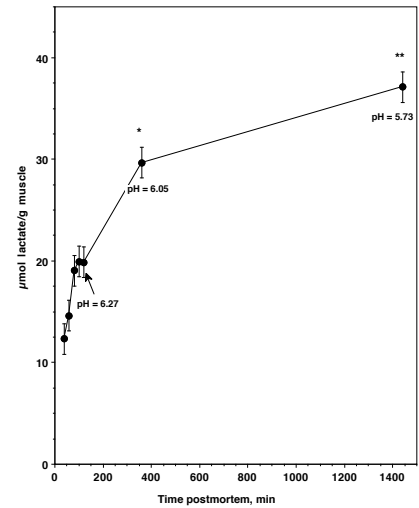
D. F6P increases, indicating inhibition at 6-PFK.



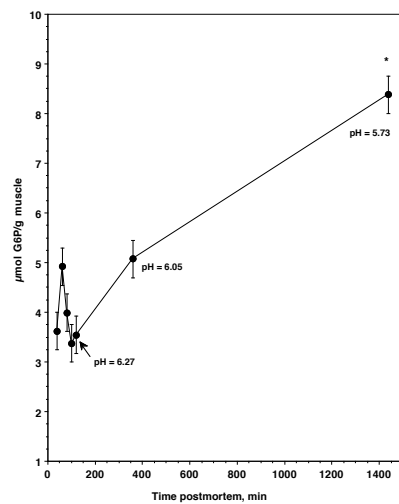
B. Glucose increases over 5-fold, caused by debranching of glycogen.



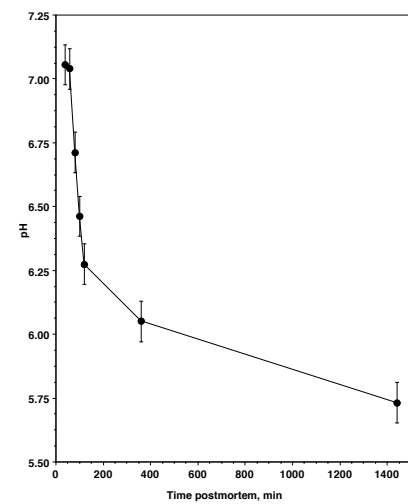
E. Lactate increases 3-fold, which causes the decline in pH.



C. G6P increases as F6P increases, which would inhibit hexokinase activity.



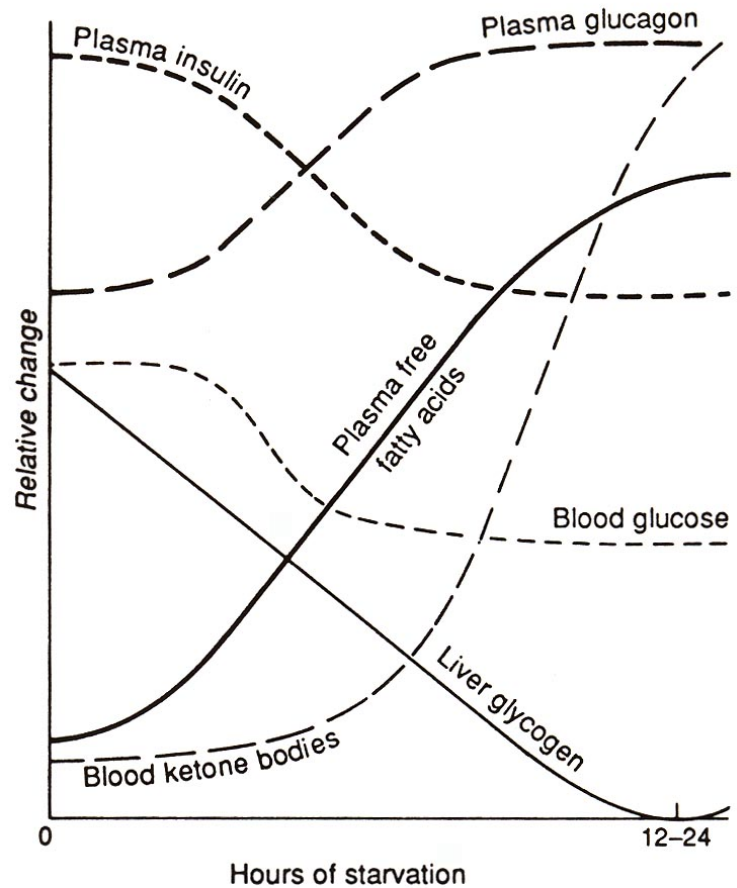
F. The pH declines to 6.75 by 4 h postmortem.



X. Effects of starvation and exercise on substrate utilization by muscle

A. During exercise or starvation/fasting:

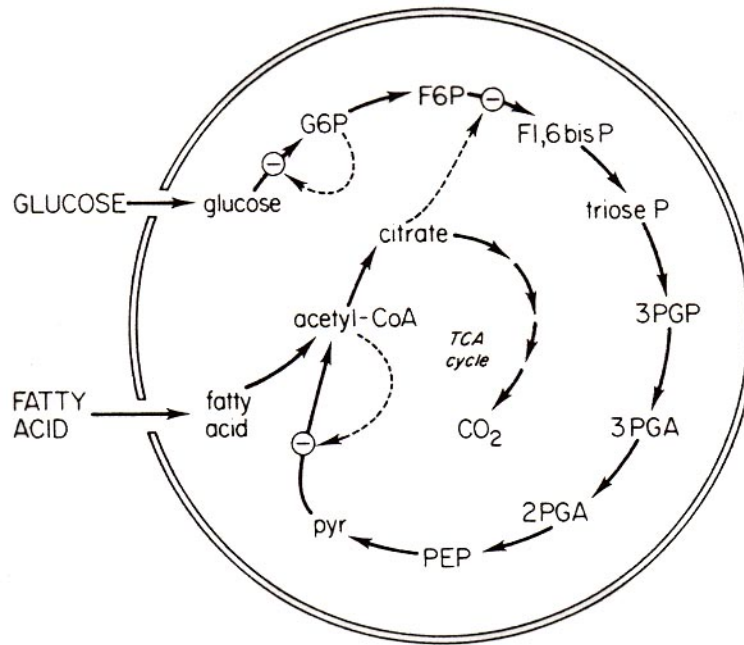
1. Liver glycogen is depleted.
2. Blood glucose decreases.
3. In response to decrease in blood glucose, insulin decreases and glucagon release from pancreas is increased.
4. Nonesterified fatty acids increase in blood.



XI. The glucose:fatty acid cycle

A. Muscle prefers ketone bodies (especially!) and fatty acids over glucose for metabolism.

1. Ketone bodies are water soluble, and are activated in mitochondria where they are metabolized (very fast).
2. The metabolism of ketone bodies and fatty acids elevates mitochondrial acetyl-CoA.
 - a. This elevates mitochondrial citrate.
 - b. Citrate exits mitochondria, elevates sarcoplasmic citrate.
3. Citrate and fatty acyl-CoAs inhibit 6-PFK.
 - a. Inhibition at 6-PFK causes increase of F-6-P and G-6-P.
 - b. Elevation of G-6-P inhibits hexokinase, spares glucose for other tissues.



XII. Muscle glycogen concentrations in biceps femoris muscle of horses before and after an exercise test

A. Fat feeding increases muscle glycogen in horses.

1. Metabolism of fat decreases glycolysis.
2. Excess glucose carbon is used to synthesize glycogen.

B. Following four gallops, fat-fed horses used nearly twice as much muscle glycogen as control horses.

C. Nearly 50% of the initial muscle glycogen remained in both control and fat-fed horses.

Diet	Pre-test	Post-test ¹	Amount used
Control	15.77 ± 0.52 n = 5	8.78 ± 1.40 n = 4	6.99 ± 0.75 n = 4
Fat supplemented (10% fat)	22.89* ± 0.42 n = 6	9.81 ± 0.81 n = 6	13.08* ± 0.43 n = 6

*Different from control

¹The exercise test consisted of four, 600 m gallops, interspersed with 5 min resting intervals. The test was performed at a speed to increase heart rates to 210 beats per minute. The horses were accelerated as fast as possible. From Oldham et al. (1990).

XIII. Epinephrine and glycogen turnover in humans

A. Infusion of epinephrine in humans causes a rapid rise in phosphorylase_a (active phosphorylase) and a rapid decline in glycogen synthase I (active glycogen synthase).

1. Caused by activation of protein kinase A and phosphorylase kinase.
2. Effects are reversed soon after epinephrine infusion ceases.
 - a. Phosphodiesterase converts cAMP to AMP.
 - b. Regulatory subunit of protein kinase binds to the catalytic subunit.
 - c. This inactivates protein kinase A.
 - d. Insulin also activates phosphatases, which remove phosphoryl groups and inactivates phosphorylase kinase and glycogen phosphorylase.

B. However, there is only a small change in muscle glycogen.

1. Free glucose accumulates in muscle.
2. Free glucose inhibits phosphorylase_a.

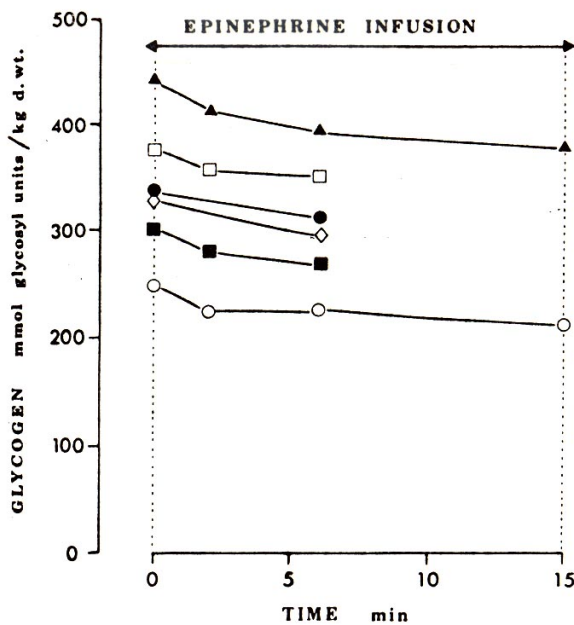


FIG. 3. Muscle content of glycogen during epinephrine infusion. Each symbol represents values from same subject. Four subjects (■, ▲, □, ○) are same as in Fig. 2.

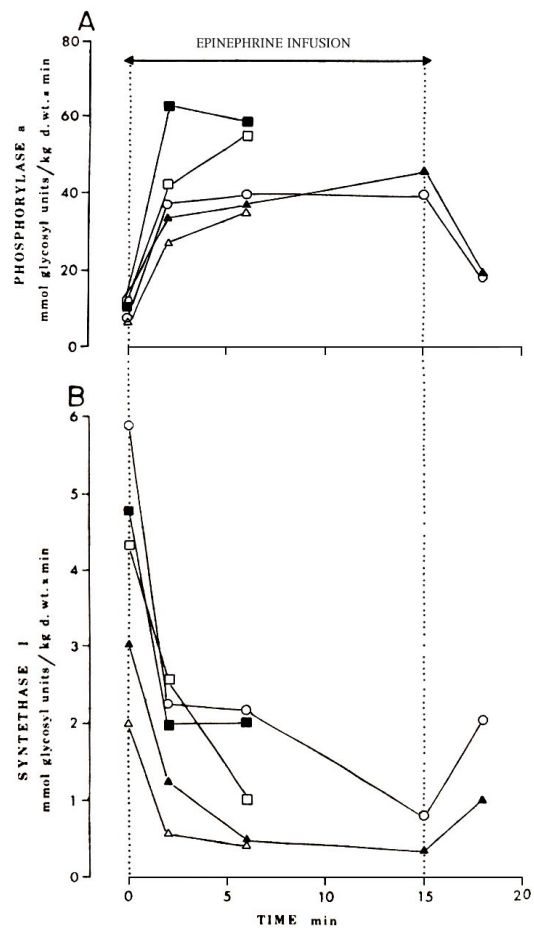


Figure A/B: Effect of epinephrine infusion on phosphorylase a and synthase activity in muscle. Each symbol represents values from same subject.

XIV. Epinephrine and glycogen turnover in cattle

- A. Starvation decreases glycogen in bovine muscle.
- B. Repletion requires several days.
- C. Electrical stimulation of muscle has no significant effect on muscle glycogen, although epinephrine significantly reduces muscle glycogen.

