Name: _____ **SS#:** _____

Section	Possible Points	<Score>
1-Rates of enzymatic reactions	**10**	5
2-Enzymatic catalysis	**10**	2
3-Sugars and polysaccharides	**10**	3
4-Introduction to metabolism	**10**	7
5-Glycolysis	**10**	5
6-Glycogen metabolism	**10**	7
7-Citric acid cycle	**10**	4
8-Electron transport	**10**	4
9-Other pathways	**10**	5
10-Photosynthesis	**10**	2
Total	**100**	43

Useful Information

$$v = \frac{V_{max} \cdot [S]}{K_m \cdot \left(1 + \frac{[I]}{K_{ic}}\right) + [S] \cdot \left(1 + \frac{[I]}{K_{iu}}\right)}$$

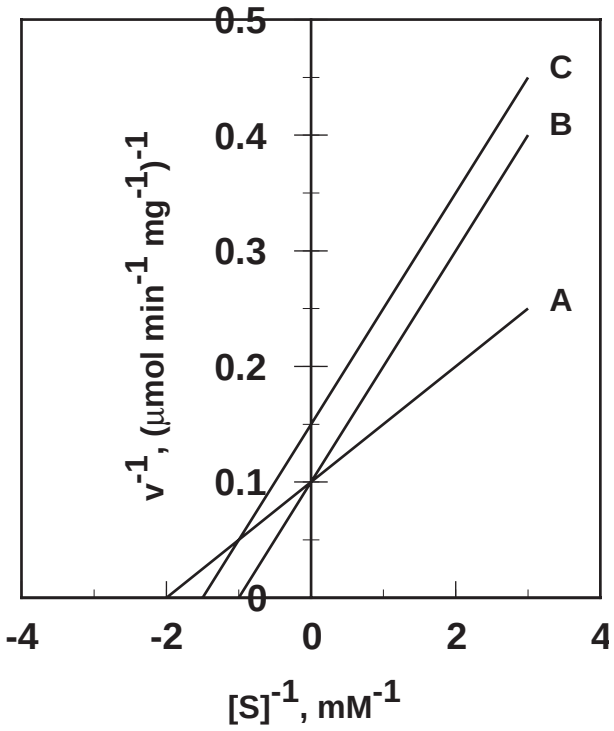
$$K_m^{app} = K_m\left(1 + \frac{[I]}{K_{ic}}\right) \qquad V_{max}^{app} = \frac{V_{max}}{1 + \frac{[I]}{K_{iu}}}$$

Compound	$\Delta G^{\circ\prime}$ (kJ·mol^{-1})
Phosphoenolpyruvate	-61.9
1,3-Bisphosphoglycerate	-49.4
Acetyl phosphate	-43.1
Phosphocreatine	-43.1
PP_i	-33.5
ATP ($\rightarrow$ AMP + PP_i)	-32.2
ATP ($\rightarrow$ ADP + P_i)	-30.5

1 *F*(faraday) = 96,485 C mol^{-1} = 96,485 J V^{-1} mol^{-1}

$$\Delta G^{\circ\prime} = -nF\Delta\varepsilon^{\circ\prime}$$

1-Rates of enzymatic reactions



The value of K_m for the enzyme depicted by curve A is

(each part: VV p. 369 related to Problem 4)

The x-intercept for curve A is $-(K_m)^{-1}$ so $K_m = -(-2\ mM^{-1})^{-1} = 0.5$ mM. (1 pt)

The value of V_{max} for the enzyme depicted by curve A is

The y-intercept for curve A is $(V_{max})^{-1}$ so $V_{max} = (0.1\ \mu mol^{-1}\ min\ mg)^{-1} = 10\ \mu mol\ min^{-1}\ mg^{-1}$ (1 pt)

Curve B depicts the effect of an inhbitor on the system described by curve A. Explain which kind of inhibitor is operating here.

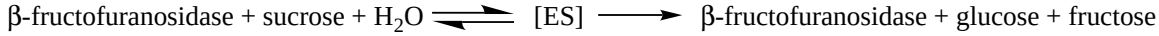
A competitive inhibitor is operating because there is an apparent increase in K_m with no change in V_{max}. (2 pts)

Curve C depicts the effect of a different inhibitor on the system described by curve A. Explain which kind of inhibitor is operating here.

A mixed inhibitor is operating because there is an apparent increase in K_m, decrease in V_{max}, and increase in K_m/V_{max} with $K_{ic} < K_{iu}$. (2 pts)

Consider the mechanisms of the following two enzyme-catalyzed reactions:

(A)



(B)

$$\text{reduced-laccase} + O_2 \rightarrow \text{oxidized-laccase}$$

$$\text{oxidized-laccase} + \text{hydroquinone} \rightarrow \text{reduced-laccase} + \text{quinone}$$

Explain briefly why the mechanism in (A) accounts for the ability of substrate to saturate the enzyme, but the mechanism in (B) accounts for the inability of substrates (held in a constant ratio) to saturate the enzyme.

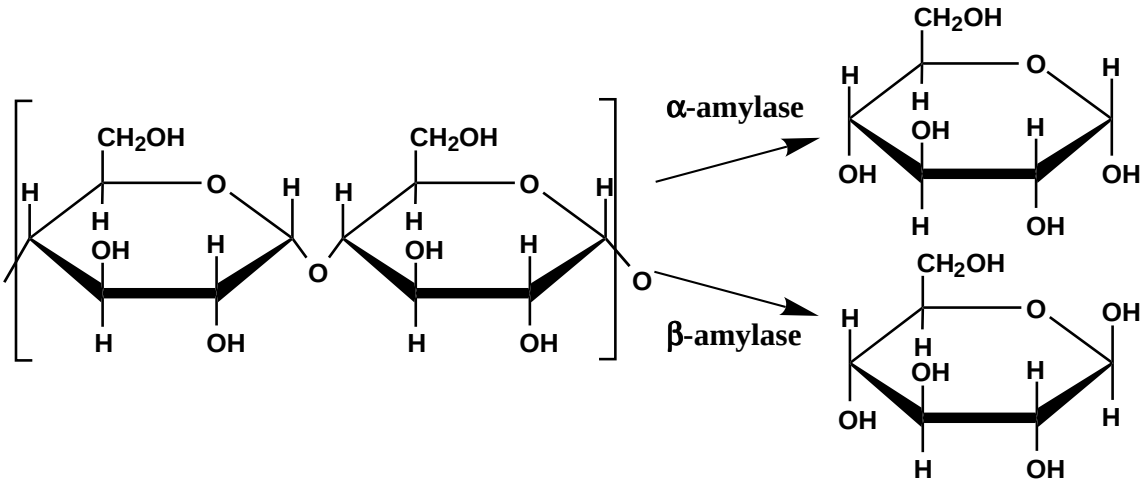
(VV pp. 346-347, 351)

In (A), the breakdown of [ES] is a first-order process and therefore at high levels of substrate, the reaction approaches zero-order. (2 pts)

In (B), there are no first-order steps, so theoretically, there will be no limitation on reaction rate. (2 pts)

2-Enzymatic catalysis

The progressive hydrolysis of the α(1→4) glucosidic bonds of amylose is catalyzed both by α-amylase and by β-amylase. In the case of α-amylase, the newly formed reducing group has the same α-configuration (before mutarotation) as the corresponding linkage in the polymer, whereas in the case of β-amylase it has the β-configuration.



Suggest reasonable mechanisms for the two group-transfer reactions that would account for these observations.

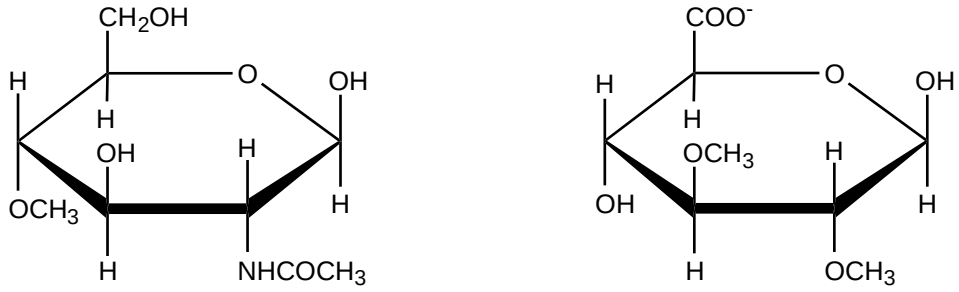
(VV pp. 385-387)

Simple bimolecular substitution reactions (called S_N2 reactions in textbooks on organic reaction mechanisms) commonly proceed with inversion of configuration at the substituted atom. Retention, as with α-amylase, can occur as a consequence of two succesive substitutions, as in a substituted-enzyme or double-displacement mechanism. Some enzymes protect or restrict access to substrates, which may result in retention of configuation. (5 pts)

Net inversion, as with β-amylase, suggests an odd number of inversions, as in a ternary-complex or single-displacement mechanism. Some enzymes protect or restrict access to substrates, which may result in inversion of configuation. (5 pts)

3-Sugars and polysaccharides

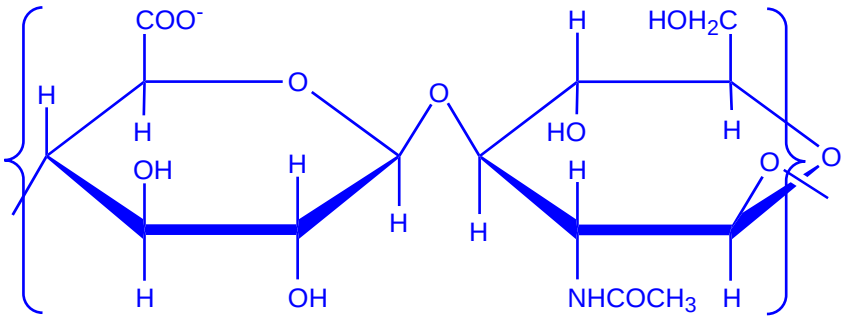
A glycosaminoglycan was isolated from a human source and exhaustively methylated. The resulting product was digested with hyaluronidase and then hydrolyzed with acid to give equimolar amounts of the following products:



Draw the structure of the isolated mucopolysaccharide and provide its name and that of the individual residues.

(VV p. 264-265. related to p. 276 Problem 12)

Methylation protects non-glycosidic bonds and hyaluronidase hydrolyzes β(1→4) linkages. Hyaluronic acid fulfills the description and determines the order of the individual residues.



D-Glucuronate **N-Acetyl-D-glucosamine**

Hyaluronate

(5 pts for the structure and 2.5 pts for each correct name)

4-Introduction to metabolism

In aerobic metabolism, glucose is completely oxidized in the reaction

$$\textbf{Glucose} + 6O_2 \rightleftharpoons 6CO_2 + 6H_2O$$

with the coupled generation of 38 ATP molecules from 38 ADP + 38 P_i. Assuming the ΔG for the hydrolysis of ATP to ADP and P_i under intracellular conditions is –48.1 $kJ \cdot mol^{-1}$ and that for the combustion of glucose is -2823.2 $kJ \cdot mol^{-1}$, what is the efficiency of the glucose oxidation reaction in terms of free energy sequestered in the form of ATP? What is the total potential number of moles of ATP available from the combustion of glucose if the process were 100% efficient?

(VV p. 442 Problem 6)

Efficiency = $38(-48.1 \text{ kJ mol}^{-1}/-2823.2 \text{ kJ} \cdot \text{mol}^{-1}) \times 100 = 65\%$ (5 pts)

Total potential ATP = $-2823.2 \text{ kJ mol}^{-1}/-48.1 \text{ kJ} \cdot \text{mol}^{-1} = 58$ (5 pts)

5-Glycolysis

When glucose is degraded anaerobically via glycolysis there is no overall oxidation or reduction of the substrate. The fermentation reaction is therefore said to be "balanced." The free energy required for ATP formation is nevertheless obtained from favorable electron-transfer reactions. Which metabolic intermediate is the electron donor and which is the electron acceptor when glucose is degraded by a balanced glycolytic fermentation: (a) in muscle and (b) in yeast? Indicate what happens to the donor and acceptor in each case.

(VV p. 482 Problem 5)

(a) The electron donor is glyceraldehyde-3-phosphate whose aldehyde group becomes oxidized to a carboxyl group in 1,3-bisphosphoglycerate. The electron acceptor is pyruvate whose ketone group is reduced to a secondary alcohol in lactate. (5 pts)

(b) The electron donor is glyceraldehyde-3-phosphate, as in (a). The electron acceptor is acetaldehyde, which is reduced to ethanol. (5 pts)

6-Glycogen metabolism

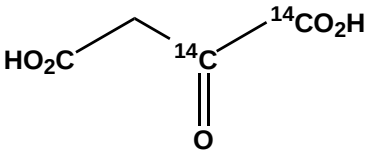
Caffeine inhibits cAMP phosphodiesterase. Explain how this affects metabolic responses to epinephrine?

(VV pp. 497-504, related to p. 512 Problem 3)

Epinephrine binding to its receptor stimulates production of cAMP and therefore promotes phosphorylation catalyzed by cAPK. When cAMP phosphodiesterase is inhibited, [cAMP] remains high and thereby prolongs the effects of epinephrine. Glycolysis is stimulated and produces more energy beyond what would normally be expected. (10 pts)

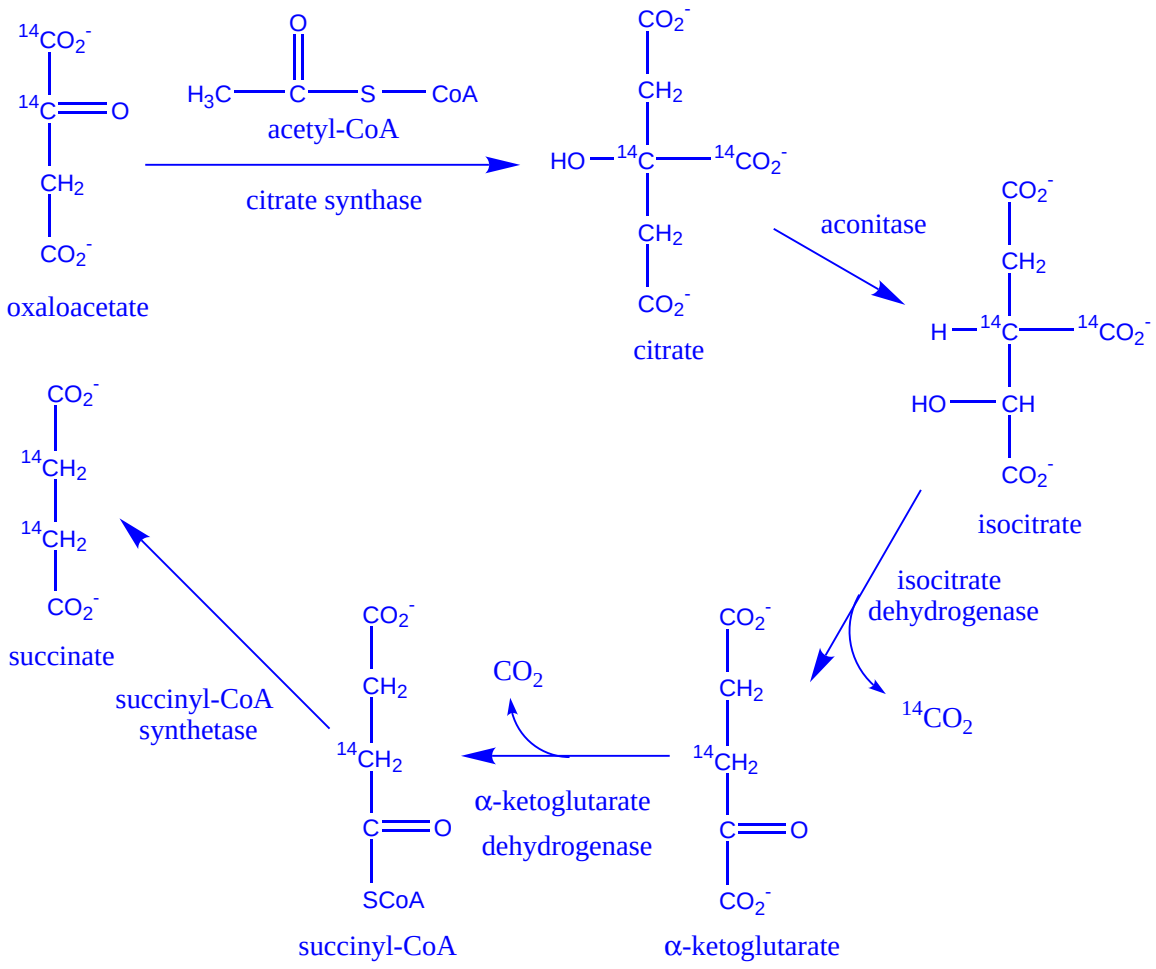
7-Citric acid cycle

An in vitro system that contained all of the citric acid cycle substrates, coenzymes, enzymes and cofactors necessary for the synthesis of succinate from oxaloacetate, but not from malate, was first totally depleted of oxaloacetate. Then ^{14}C-labeled oxaloacetate was added with the label located and distributed equally as shown below. Determine the distribution of ^{14}C in the synthesis of succinate.



(VV p. 539, related to p. 562 Problem 1)

Fifty percent of the ^{14}C label will be lost as CO_2 as a result of isocitrate dehydrogenase. (5 pts)
Fifty percent of the ^{14}C label will be equally distributed between C2 and C3 of succinate.



(5 pts)

8-Electron transport

Oligomycin and cyanide both inhibit oxidative phosphorylation when the substrate is either pyruvate of succinate. Dinitrophenol can be used to distinguish between these inhibitors. Explain.

(VV p. 598 Problem 9)

Oligomycin inhibits F_1F_o-ATPase while CN^- inhibits cytochrome oxidase. Because electron transport through cytochrome oxidase is coupled with ATP synthesis, both inhibitors inhibit the oxidative phosphorylation of pyruvate and succinate. Dinitrophenol uncouples oxidative phosphorylation so that substrate oxidation can occur in the absence of ATP synthesis. Oligomycin and CN^- inhibition may be distinguished by measuring O_2 uptake in the presence of dinitrophenol. Oligomycin does not inhibit O_2 uptake in the presence of dinitrophenol while CN^- does so. (10 pts)

9-Other pathways

Compare the relative energetic efficiencies, in ATPs per mole of glucose oxidized, of glucose oxidation via glycolysis plus the citric acid cycle versus glucose oxidation via the pentose phosphate pathway plus gluconeogenesis. Assume that NADH and NADPH are each energetically equivalent to three ATPs and $FADH_2$ is energetically equivalent to two ATPs.

(VV p. 625 Problem 1)

The formation of glucose-6-phosphate from glucose via the hexokinase reaction requires the expenditure of 1 ATP. The pentose phosphate pathway generates 2NADPH = 6ATP for each CO_2 released. The resulting ribulose-5-phosphate can be reconverted to glucose-6-phosphate through the remaining reactions of the pentose phosphate pathway and gluconeogenesis (F6P $\rightarrow$ G6P and GAP $\rightarrow$ DHAP $\rightarrow$ FBP $\rightarrow$ F6P $\rightarrow$ G6P) without further input of energy. Thus, conversion of glucose to CO_2 via the pentose phosphate pathway plus gluconeogenesis generates (6 x 6) - 1 = 35ATP. (5 pts)

Glycolysis converts 1glucose to 2pyruvate + 2ATP + 2NADH. The 2pyruvate are converted to 2acetyl-CoA + 2NADH by pyruvate dehydrogenase. The citric acid cycle converts 2acetyl-CoA to 6NADH + 2GTP + $2FADH_2$ + $4CO_2$. Hence, altogether this process generates 2ATP + 10NADH + $2FADH_2$ + 2GTP. Because each NADH = 3ATP, each $FADH_2$ = 2ATP, and each GTP = ATP, this is equal to 2 + (3 x 10) + (2 x 2) + 2 = 38ATP. (5 pts)

10-Photosynthesis

Why is it possible for chloroplasts to absorb much more than 8-10 photons per O_2 molecule evolved?

(VV p. 645)

When cyclic electron flow occurs, photoactivation of PSI drives electron transport independently of the flow of electrons derived from water. Thus, the oxidation of H_2O by PSII is not linked to the number of photons consumed by PSI. (10 pts)