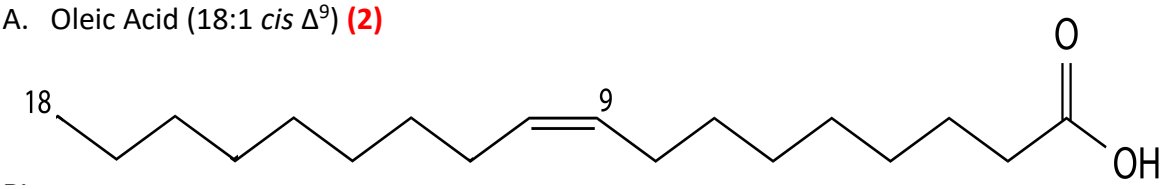
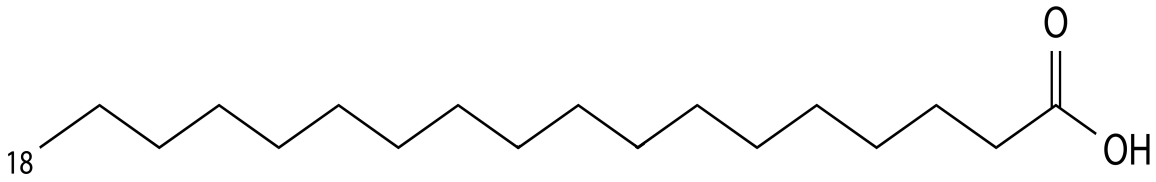
Lipids / FA & Chol metabolism :

1. **Draw the following fatty acids, and arrange the names below from highest melting point (1) to lowest melting point (4).**

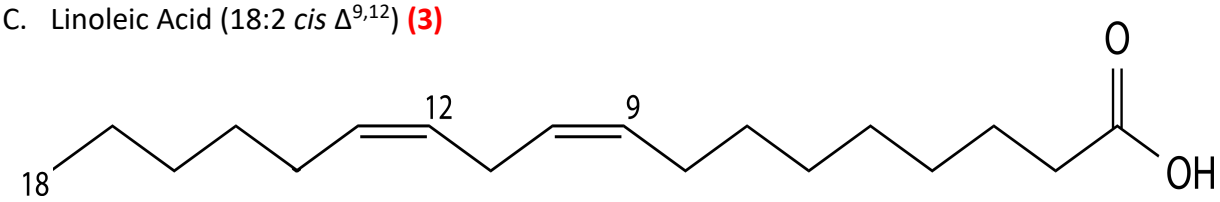
   A. Oleic Acid (18:1 *cis* Δ^{9}) **(2)**



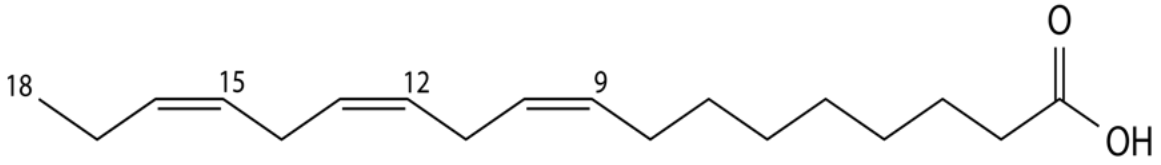
   B. Stearic Acid (18:0) **(1)**



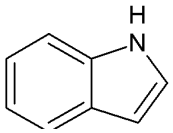
   C. Linoleic Acid (18:2 *cis* $\Delta^{9,12}$) **(3)**



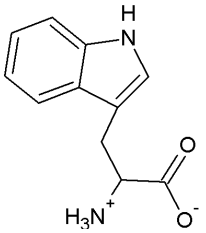
   D. Linolenic Acid (18:3 *cis* $\Delta^{9,12,15}$) **(4)**



2. **Tryptophan has a pka of 5.89 while Indole has a pka higher than 10. At pH 7, tryptophan crosses a lipid bilayer at about one-thousandth the rate of indole. Suggest an explanation for this observation.**



Indole



Tryptophan

Funtional groups are deprotonated at pH higher than pka and protonated at pH lower than the pka. Trp has a pka of 5.89 while indole has a pka higher than 10 → Indole is uncharged at pH 7 while tryptophan is charged and so it cannot pass through hydrophobic interior easily.

3. **Membrane lipids in tissue samples obtained from different parts of the leg of a reindeer show different fatty acid compositions. Membrane lipids from tissue near the hoof contain a larger proportion of unsaturated fatty acids than lipids from tissue in the upper part of the leg. Comment on the significance of this observation.**

Hooves are exposed to the snow and therefore a colder environment. To maintain membrane fluidity, the fatty acids must have a lower melting temperature, achieved by more unsaturated fatty acids.

4. **Choose from the following list below and pick the correct options to fill in the blanks in the statements below. Note: Not all terms will be used!**

Glycerophospholipids	12
Cholesterol	14
Sphingomyelin	16
Coenzyme A	FADH2
Vitamin B6	NADH
Vitamin E	Thromboxanes
Eicosanoids	Insulin
Chylomicrons	Ketone bodies
Bile salts	Estrogen
Fat globules	Glucagon

i. Cholesterol is an amphipathic molecule with planar and rigid structure that forms a pre-cursor for steroid hormones.

ii. The carnitine shuttle is required to bring fatty acids into the mitochondrial matrix (from the cytosol) when their chain length is 14 carbons or greater.

iii. Bile salts released in the small intestines help in breaking down fats into smaller emulsion droplets to allow better access for **water soluble lipases** to begin breaking down the TAG into free FA's and glycerol.

iv. Chylomicrons are composed of TAGs, phospholipids, apolipoproteins and cholesterol.

v. The coenzyme required for all transaminations is derived from vitaminB6.

vi. The starvation signal glucagon triggers a series of events that releases TAGs from adipose tissues.

vii. Vital organs like the brains and heart rely on ketone bodies are their source of energy during a state of starvation.

viii. Breakdown of a C16:1 fatty acid yields 7 NADH and 6 FADH2 at the end of the 7 cycles of b-oxidation.

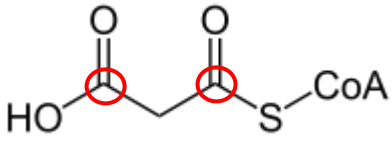
5. **In a laboratory experiment, two groups of rats are fed two different fatty acids as their sole source of carbon for a month. The first group gets heptanoic acid (C7:0), and the second gets octanoic acid (C8:0). After the experiment, a striking difference is seen between the two groups. Those in the first group are healthy and have gained weight, whereas those in the second group are weak and have lost weight as a result of losing muscle mass. What is the biochemical basis for this difference?**

Group1: Beta-oxidation of odd chain FAs can generate propionyl CoA which can feed into the TCA cycle as succinyl CoA to generate OAA. The OAA generated here can be siphoned off to make glucose via gluconeogenesis.

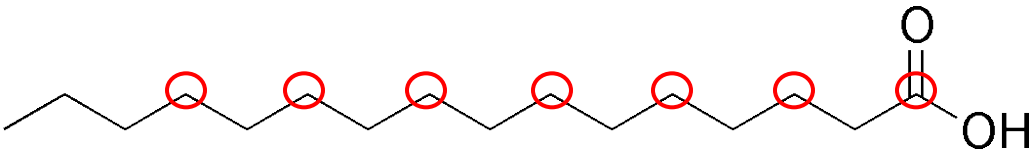
Group 2: Beta-oxidation of even chain FA can only generate acetyl-CoA. When acetyl-CoA feeds into the TCA cycle, there is not net gain of OAA and hence it cannot be siphoned out to other biochemical reactions such as gluconeogenesis to make glucose. In cases like this, OAA is usually obtained from protein breakdown (in muscle tissue) leading to loss of weight and muscle mass.

6. **Malonyl CoA is 14C-radiolabeled in C1 and C3 position, and is added into the cytosol for fatty acid synthesis.**

A. **Draw the structure of malonyl CoA and circle the radio-labeled atoms.**



B. Fatty acid synthase has completed its synthesis. Draw the final fatty acid product and circle the radio-labeled atoms.



7. How many C22:0 fatty acids are required to supply 12,000 ATP? Also calculate the number of water molecules generated from the oxidation of the fatty acids required above.

Activation cost = 2 ATP used
10 rounds of beta-oxidation → (10 X NADH) + (10 X $FADH_2$) = 40 ATP
11 Acetyl-CoA → TCA cycle = 110 ATP
Each fatty acid = 148 ATP
12,000 / 148 = 81.08 = ~ 82 fatty acids required

10 rounds of beta-oxidation → (10 X NADH) + (10 X $FADH_2$) – 10 hydration rxns = 10 water molecules
11 Acetyl-CoA → TCA cycle = 22 water molecules
Each fatty acid = 32 water molecules
32 water molecules X 82 fatty acid molecules = 2624 water molecules

8. Compare and contrast fatty acid synthesis and their breakdowns. Consider their:

	Synthesis	Breakdown
Activating Group	**ACP**	**CoA**
Electron carrier coenzyme	**NADPH**	**FAD & NAD+**
Basic units added or removed	**Acetyl (malonyl)**	**Acetyl**
Cellular Location	**Cytosol**	**Mitochondrial matrix**

9. The synthesis of palmitate by fatty acid synthase (FAS) has a net production of 6 water molecules even though there are a total of 7 dehydration reactions involved during the synthesis process. Explain.

After the last round of synthesis, the 16 carbon acyl chain is still attached to ACP via a thioester linkage. Thioesterase uses 1 molecules of water as a substrate to hydrolyze palmitoyl-ACP and release palmitate.

10. **Researchers are monitoring the enzymatic activities of several key enzymes in your body involved in fat metabolism. Phosphorylation is a very common reversible process used to regulate metabolic pathways. For the following enzymes, suggest when the researchers would find them in a phosphorylated state. *i.e.* during times of starvation or times of plenty? Briefly comment the effect of this phosphorylation.**

Hormone sensitive lipase – **starvation (mobilize fats, triggered by glucagon)**

Acetyl CoA carboxylase – **starvation (inactivates/inhibit committed step of FA synthesis)**

Carnitine acyltransferase I – **not phosphorylated (committed step in beta-oxidation)**

HMG-CoA reductase – **starvation (inactivates/inhibit comm step in cholestersol syn)**

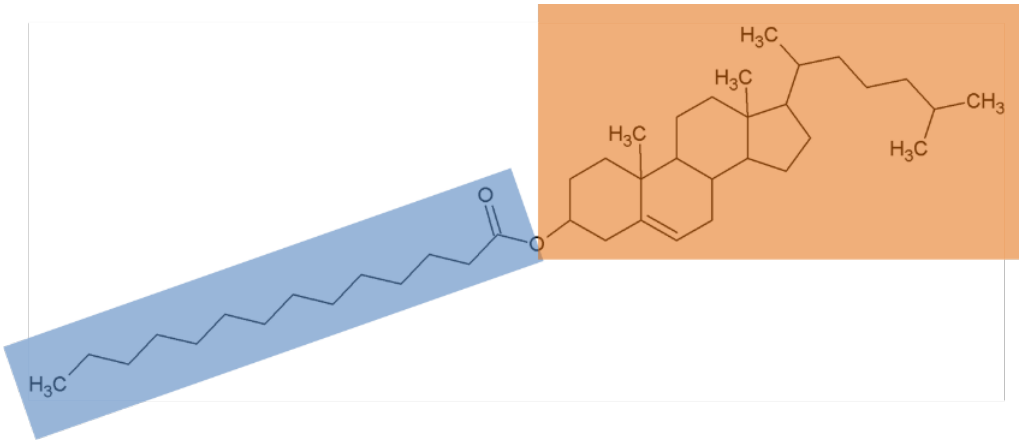
11. **In your desire to ace BIOC 302, you have locked yourself in your bedroom so that you cannot get out until you have learnt all the material. Unfortunately, it's harder than you thought, and you've been locked in the room for two days. Which of the following reactions are happening in your body?**

    A. **High carnitine acyltransferase I activity** – True, since b-oxidation is upregulated.
    B. **Dephosphorylation of ACC** – False, since FA synthesis is downregulated.
    C. **Activation of HMG-CoA lyase** – True, since ketone body synthesis is upregulated.
    D. **Increased activity of citrate lyase** – False, since FA synthesis is downregulated.
    E. **Increased phosphorylation of perilipin** – True, since FA mobilization is upregulated.
    F. **Activation of HMG-CoA reductase** – False, since chol biosynthesis is downregulated.

    a. A and E
    b. B and C
    c. D and E
    d. E and F
    e. B, E and F
    f. A, C and E
    g. None of the above

12. Esterification is a very common modification made to cholesterol.

A. Draw the structure of cholesterol and cholesterol esterified to a myristic acid. (C14:0)



B. Compare the above structures and consider the morphology of lipoproteins. Why are only cholesteryl esters packaged into the interior of lipoproteins but not cholesterol?

Esterification removes polar headgroup from cholesterol, converting the amphipathic molecule to a hydrophobic molecule. This allows them to be packaged into the hydrophobic interior of lipoproteins.

C. Lecithin Cholesterol Acyl Transferase (LCAT) catalyses the formation of cholesteryl ester with the production of a phospholipid by-product. Speculate on how this phospholipid by-product contributes to the morphology of a lipoprotein.

LCAT cleaves an acyl chain off the structure of lecithin, forming lysophosphatidylcholine. This has an overall inverted cone shape and can form micelles.

13. Suppose an individual is undergoing severe starvation. This individual's liver cell contains 32 molecules of C18:1 cisΔ^9 and 26 molecules of C16:0. In this particular liver cell, ALL of the fatty acids undergo beta oxidation and all of the resulting products are used for ketone body synthesis.

A. Why is the liver <u>unable</u> to catabolize ketone bodies?

Liver does not express b-ketoacyl-CoA enzyme required to catabolize ketone bodies.

B. What is the maximum number of B-hydrobutyrate molecules that could potentially be synthesized from 32 molecules of C18:1 cisΔ^9 and 26 molecules of C16:0 in the liver cell after b-oxidation occurred?

32 molecules 18C long → 576 carbons
26 molecules 16C long → 416 carbons
➔ 992 carbons used to generate acetyl-CoA
#acetyl-CoA = 992/2 = 496
#b-hydroxybutyrate = 496/2 = 248

C. Using your answer from part B, assume all of these b-hydroxybutyrate molecules are shipped via the bloodstream to the heart. If the heart completely catabolizes the maximum number of b-hydroxybutyrate molecules to CO2 and H2O, how many ATP equivalents are generated? Assume that the enzyme b-ketoacyl COA transferase uses -1 ATP equivalent per reaction and that the heart has adequate OAA.

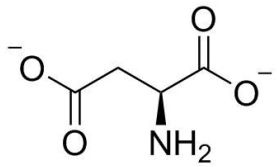
248 b-hydroxybutyrate + 248 NAD+ → 248 acetoacetate + 248 NADH + 248 H+ ➔ 620 ATP
248 acetoacetate → 248 acetoacetyl-CoA ➔ b-ketoacyl COA transferase uses -1 ATP equivalent per reaction = – 248 ATP
248 acetoacetyl-CoA → 496 Acetyl-CoA → TCA cycle ➔ 4960 ATP
Net ATP gain: 5332 ATP

Amino acids/ Protein metabolism:

14. Castor beans can be processed and purified to produce ricin, a highly toxic protein that fully inhibits protein synthesis. Explain why after exposure to ricin, it takes a few hours to a full day for death to occur.

Protein turnover → Immediately inhibiting translation has no effect as you still have active proteins. Over time, they begin to degrade and then you run into trouble.

15. The following structure can be described as an "acidic amino acid." Which amino acid is it and explain why.



This amino acid is aspartate. At pH 7, the amino group is protonated, while the carboxylic acid groups are deprotonated → this gives the molecule a net negative charge. The pKa of carboxyl group is 1.99, pKa of amino group is 9.90, pKa of R group is 3.90. Acidic amino acids are negatively charged at neutral pH, while basic amino acids are positively charged.

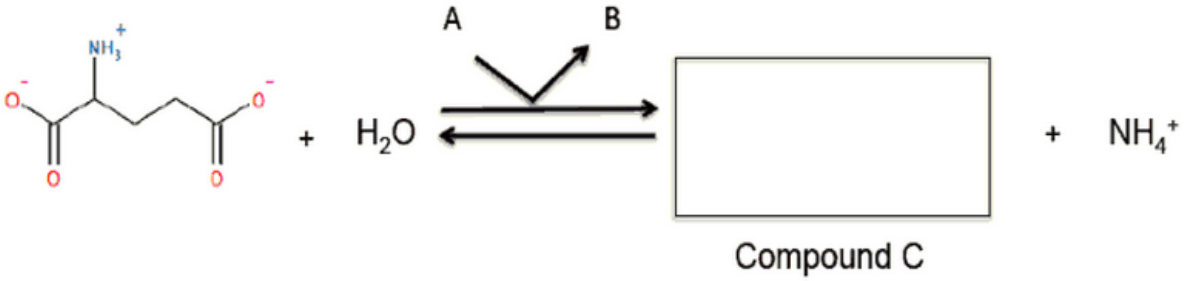
Draw the corresponding α-keto acid for the above amino acid. What is the significance of this α-keto acid

Oxaloacetate is TCA cycle intermediate, it combines with acetyl-CoA to form citrate.

16. Which of the following is a characteristic of many aminotransferase reactions?

A. They have a large, negative $\Delta G'^{o}$
B. The amino group is transferred to an α-keto acid (such as α-ketoglutarate) to form the corresponding amino acid
C. The amino group is transferred from an ammonia molecule
D. All reactants and products are identical for all aminotransferase reactions.
E. They require the cofactor S-adenosylmethionine

17. Consider the following reaction:



A. What is compound C? alpha-ketoglutarate

B. What are A and B in the reaction?

A: NAD+ or NADP+, B: NADH or NADPH (**assume it is NADH for calculation purposes)

C. What enzyme catalyzes this reaction? glutamate dehydrogenase

D. Name a cell type and a cellular location in mammals where the above reaction takes place. Liver mitochondria matrix

E. How many ATP equivalents would be produced from oxidation of compound C in the TCA cycle? 7.5ATP

18. **Calculate the ATP cost (or gain) for removal of nitrogenous waste in the following two scenarios. (Do not assume oxidation of the carbon skeleton in this question – just think about the nitrogenous waste removal)**

- **1st Scenario: Alanine from an extrahepatic cell is transported to the liver mitochondrial matrix to remove its α-amino group. The α-amino group enters the urea cycle to produce urea. Assume that abundant amounts of aspartate are available.**

  - Alanine is used transported to the liver through the blood stream
  - Alanine undergoes an aminotransferase reaction and transfers its α-amino group onto α-Kg to form glutamate
  - Glutamate enters the mitochondrial matrix and goes through the glutamate dehydrogenase reaction to release the ammonia → **+ 2.5 ATP**
  - Free ammonia goes through the CPS 1 reaction to make carbamoyl phosphate → **– 2 ATP**
  - urea cycle:
    - Argininosuccinate synthetase → **– 2 ATP**
    - Fumarate through the TCA cycle → **+ 2.5 ATP**
  - **Net ATP gain → + 1 ATP**

- **2nd Scenario: Glutamate in an extrahepatic cell is converted to glutamine. Glutamine is transported to the liver mitochondrial matrix where BOTH the α-amino group and the side-chain amino group enter the urea cycle to produce urea. Assume that abundant amounts of aspartate are available.**

  - Glutamate is converted to glutamine in extra hepatic cells → **– 1 ATP**
  - Glutamine is transported through the blood stream and enter the liver mitochondrial matrix
  - Glutamine is converted back into Glutamate by releasing an $NH4^+$ molecule (side chain amino group)
  - Free ammonia goes through the CPS 1 reaction to make carbamoyl phosphate → **– 2 ATP**
  - [Asp] is present in abundant amounts so there is no need to make more.

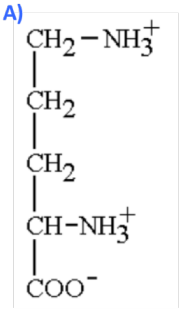
- Glutamate goes through the glutamate dehydrogenase reaction to release the ammonia molecule (a-amino group) → **+ 2.5 ATP**
- Free ammonia goes through the CPS 1 reaction to make carbamoyl phosphate → **– 2 ATP**
- Urea cycle: takes place twice since two ammonia molecules go through the CPS 1 reaction
    - Argininosuccinate synthetase X 2 → **– 4 ATP**
    - Fumarate through the TCA cycle X 2 → **+ 5 ATP**
    - 2 molecules of urea are formed.
- **Net ATP cost → – 1.5 ATP**

3rd Scenario: 2 Glutamate molecules enter the urea cycle and [Asp] concentration in the liver is low.

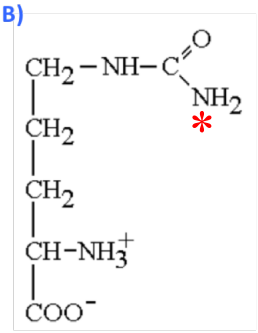
- 1 Glutamate enters the mitochondrial matrix and goes through the glutamate dehydrogenase reaction to release the ammonia → **+ 2.5 ATP**
- Free ammonia goes through the CPS 1 reaction to make carbamoyl phosphate → **– 2 ATP**
- [Asp] is low so the 2nd Glutamate has to feed into the Asp aminotransferase → **0 ATP**
- urea cycle:
    - Argininosuccinate synthetase → **– 2 ATP**
    - Fumarate through the TCA cycle → **+ 2.5 ATP**
- **Net ATP gain → + 1 ATP**

19. **Suppose that glutamate labeled with ^{15}N undergoes oxidative degradation in the liver of a rat. The amino group then enters the urea cycle to produce a molecule of urea. On which atoms of the following metabolites will the isotopes be found? Assume <u>in this case</u> that aspartate must be synthesized from glutamate and that unlabelled ornithine is already abundant. Mark ^{15}N atoms with an asterisk (*).**

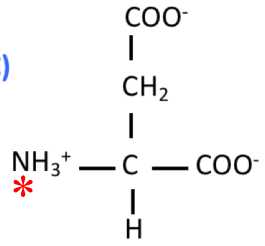
A)



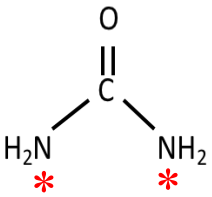
B)



C)



D)



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20. **Which of the following would be expected in a newborn infant with a deficiency in argininosuccinate synthetase (citrulline + aspartate + ATP argininosuccinate + AMP + PP_i)?**

A. Increased plasma levels of glutamine

B. Decreased plasma levels of ammonia

C. Increased plasma levels of urea

D. Decreased blood pH

E. CNS symptoms present at birth

21. **Which of the following statements about the mechanism of transamination reactions is correct?**

A. ATP hydrolysis is used to transfer the amino group on the Schiff base to the carbonyl group of keto acid acceptor.

B. The first step of a transamination reaction is the formation of a Schiff base between pyridoxal-5'-phosphate and the carbonyl group of the keto acid.

C. The first step of a transamination reaction is the formation of a Schiff base between the carbonyl group of pyridoxal-5'-phosphate and the amino group of the amino acid.

D. The first step of a transamination reaction is the formation of a Schiff base between pyridoxamine phosphate and the keto acid.

22. Which of the following enzyme reactions takes place during the synthesis of urea from ammonium ion and glutamate?

A. Carbamoyl phosphate + citrulline = ornithine

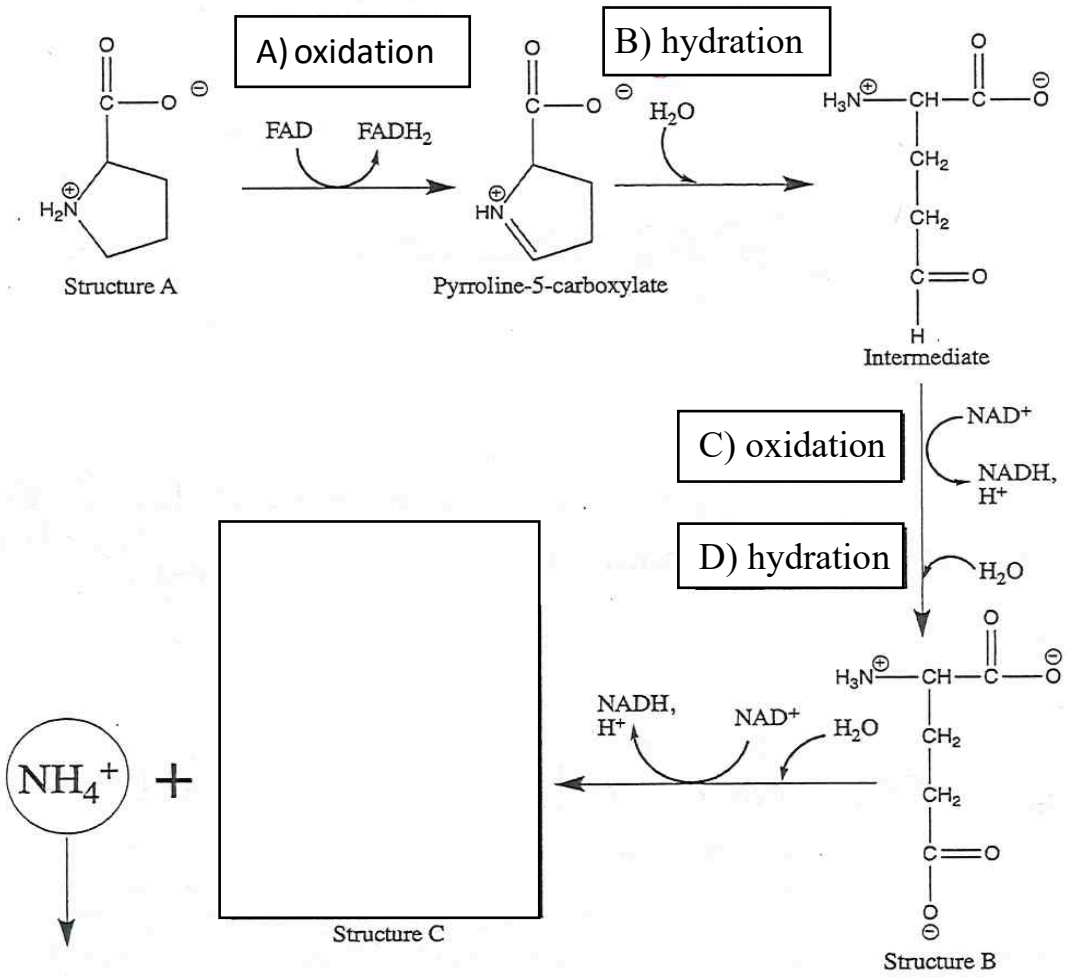
B. Aspartate + citrulline + ATP = argininosuccinate + AMP + PPi

C. Argininosuccinate = aspartate + arginine

D. CO2 + NH4+ + 2 ADP = carbamoyl phosphate + 2 ATP

E. Argininosuccinate = arginine + urea

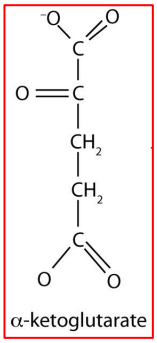
23. The catabolism of an unnamed compound (Structure A) is shown below. Study the following pathway carefully and answer the questions below.



A. Give the name of Structure A: Proline and Structure B: Glutamate

B. Have you come across the same sequence of enzymatic reactions A-B-C before? Name another pathway that utilizes the same 3-step sequence of reactions: B-oxidation

C. Structure B undergoes a chemical reaction that involves the removal of the amino group so that the amino group can enter the urea cycle. To complete this chemical reaction, please draw the chemical structure of Structure C in the box above.



D. Based on the information provided on the diagram, would you describe Structure A as glucogenic, ketogenic or both? glucogenic
Briefly explain you reasoning below:

Produces a-kg which is a TCA cycle intermediate that result in a net gain of OAA that can feed into gluconeogenesis to synthesize glucose.