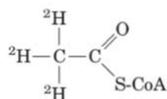


Problem Set 11 KEY

Problem 1

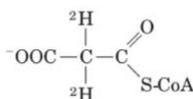
A) You are given a solution that contains all of the components necessary for fatty acid biosynthesis from acetyl-CoA and malonyl-CoA

- I) If [2- ^2H]acetyl-CoA (deuterium labeled acetyl-CoA shown below) and an excess of unlabeled malonyl-CoA are used, how many deuterium atoms are incorporated into each molecule of palmitate and what are their locations?



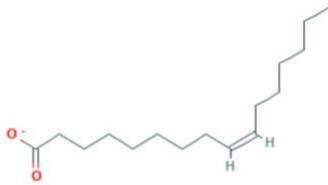
3 deuteriums will be incorporated per molecule of palmitate all located on C-16.

- II) If unlabeled acetyl-CoA and [2- ^2H]malonyl-CoA are used, how many deuterium atoms are incorporated into each molecule of palmitate and what are their locations?



7 deuteriums will be incorporated per molecule of palmitate. All will be located on even number carbons except C-16

B) Palmitoleate is a common unsaturated fatty acid with a cis double bond at Δ^9 .



Biosynthesis of Palmitoleate requires the use of palmitate as a precursor. Can this reaction be carried out in a completely anaerobic environment?

The double bond introduced in palmitoleate is formed through an oxidation reaction catalyzed by the enzyme fatty acyl-CoA desaturase. This reaction requires O₂ as a substrate, so this reaction cannot be carried out under anaerobic conditions.

Problem 2

The acetyl group from acetyl-CoA in the mitochondrion is transported into the cytosol by a shuttle system in order to carry out fatty acid synthesis in the cytosol.

A) What is the name of this shuttle? Write the overall equation for the transfer of an acetyl group from the mitochondrion to the cytosol.

Citrate-Malate Shuttle



B) How many ATPs are needed in this process per acetyl group?

1

C) One result of the carnitine shuttle (facilitates β Oxidation) is the spatial separation of cytosolic and mitochondrial pools of acetyl-CoA. Does this acetyl group shuttle also accomplish this? Why is this important?

Yes it does. As mitochondrial [Acetyl-CoA] increases, citrate is transported out of the mitochondria. In addition to acting as a precursor for acetyl-CoA and activating acetyl-CoA carboxylase, citrate inhibits phosphofructokinase-1 which reduces glycolytic flux, all of which take place in the cytosol.

Problem 3

A) In mammals, mature red blood cells lack mitochondria and must instead rely on mitochondria-independent metabolic pathways. You observe that red blood cells generate both lactate and CO_2 as products of glucose catabolism. Describe the pathway(s) used by red blood cells to produce these.

In the absence of mitochondria, ATP generation from glucose requires the regeneration of NAD^+ from NADH via reduction of pyruvate to lactate.

The pentose phosphate pathway occurs in the cytosol and generates CO_2 in the second oxidation reaction.

B) It has been proposed that rather than operating sequentially (i.e. oxidative pentose phosphate pathway-followed by non-oxidative pentose phosphate pathway), it is possible for the pentose phosphate pathway to operate as a pentose phosphate cycle. Briefly explain how it would be possible for products of the non-oxidative pentose phosphate pathway to re-enter the oxidative pentose phosphate pathway. Would such a cycle allow for the complete oxidation of glucose to CO_2 by red blood cells?

The products of the non-oxidative pentose phosphate pathway are fructose-6-phosphate and glyceraldehyde-3-phosphate. While these metabolic intermediates can be further metabolized by the glycolytic pathway, they also potentially can serve as substrates for gluconeogenesis. By converting the products of non-oxidative pentose phosphate pathway via gluconeogenesis back into glucose-6-phosphate, the non-oxidative pentose phosphate pathway can be linked to oxidative pentose phosphate pathway forming the pentose phosphate cycle. Loss of a net 1 CO_2 from each molecule of glucose-6-phosphate entering this cycle would allow for complete oxidation of glucose in the absence of mitochondria.

C) Red blood cells are continuously exposed to both endogenous and exogenous sources of reactive oxygen species like superoxide and hydrogen peroxide. What is one mechanism by which these ROS are neutralized? How might the increased use of the pathway described in A) allow red blood cells to better tolerate this oxidative stress?

Reactive oxygen species are neutralized through reduction by glutathione. The regeneration of reduced glutathione (GSH) from oxidized (GSSG) requires NADPH. Increased flux through the oxidative pentose phosphate pathway results in elevated levels of NADPH, which leads to an increased capacity to regenerate reduced glutathione, and increased ability to tolerate oxidative stress.

Problem 4

A) Which steps of the urea cycle occur in the mitochondria and which occur in the cytosol?

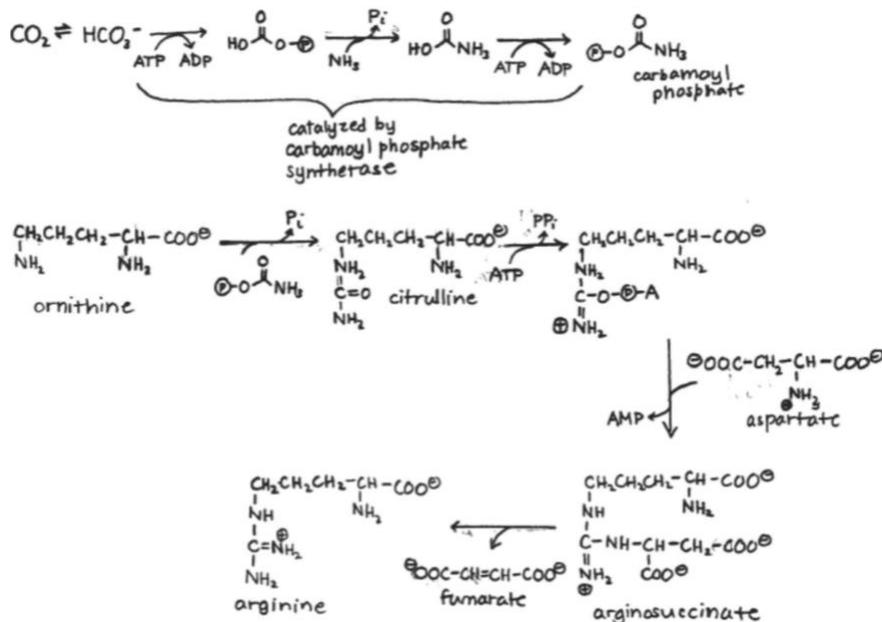
The formation of NH_4 by glutamate dehydrogenase, the generation of carbamoyl phosphate and citrulline all occur in the mitochondrial matrix.

B) Glutamine is the most abundant amino acid in the body. Why is glutamine so vital for survival? Include relevant reactions in your explanation.

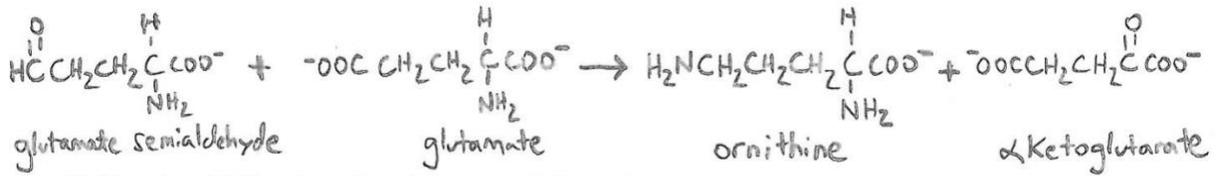
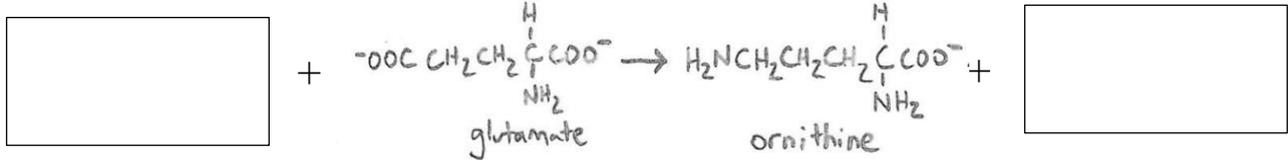
Glutamine is a nitrogen donor for all other uses of nitrogen, donating one NH_3 in its conversion to glutamate, and another in its subsequent conversion to alpha-ketoglutarate. These reactions above effectively donate nitrogen to other reactions in the body. For example, the conversion of pyruvate to alanine.

C) When arginine is not available from the environment, cells use the same reactions as those found in the urea cycle to produce arginine. Describe how cells generate arginine from the amino acids ornithine and aspartate. Include all enzyme-catalyzed steps in your answer. It is not necessary to provide enzyme names or any reaction mechanisms, but for each step be sure to indicate all of the products and reactants. Assume ATP, CO_2 and NH_3 are freely available.

Ornithine is a urea cycle intermediate that is converted to citrulline by reaction with carbamoyl phosphate, which is produced in the mitochondria by the enzyme carbamoyl phosphate synthase. Citrulline is then "primed" with an AMP, which allows aspartate to displace AMP on citrulline, forming arginosuccinate. Release of fumarate from the arginosuccinate yields arginine. All of the reactions shown are essentially the same ones as from the urea cycle.



D) To net synthesize arginine, cells require a source of ornithine that is different from the production of ornithine in the urea cycle. Ornithine is generated via transamination, via the same PLP-dependent mechanism discussed in class. Glutamate acts as the primary amine donor. What are the other reactants and products for this process (draw the chemical structures).



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7.05 General Biochemistry
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