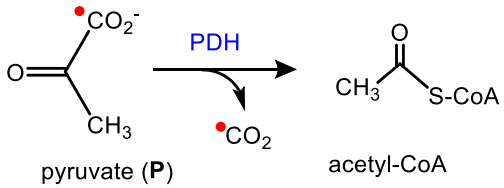
Chemistry 5.07
Problem Set 7 Answers

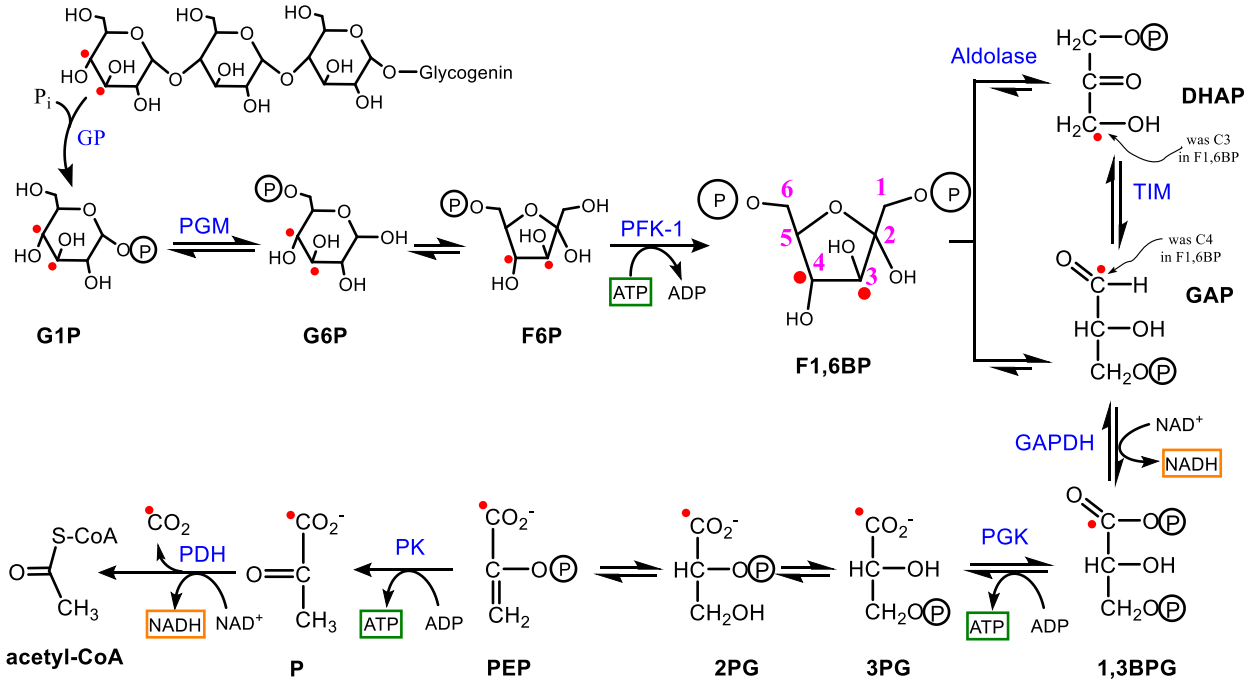
Problem 1

1. This question gives you experience tracking a labeled atom through the catabolic pathways we have studied so far.

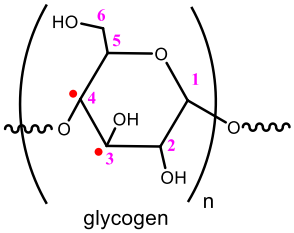
a. Carbon dioxide is lost in the pyruvate dehydrogenase step of respiration. Starting with glycogen, and working through glycolysis, please show which glycogen carbon(s) contribute to that CO_2 loss. Show pathway in sufficient structural detail so that we know that you know what you are doing.



The CO_2 lost in the PDH step (above) is marked with a red dot. You can work your way backwards (starting from the bottom of the page) through the glycolysis pathway all the way to G6P and glycogen.

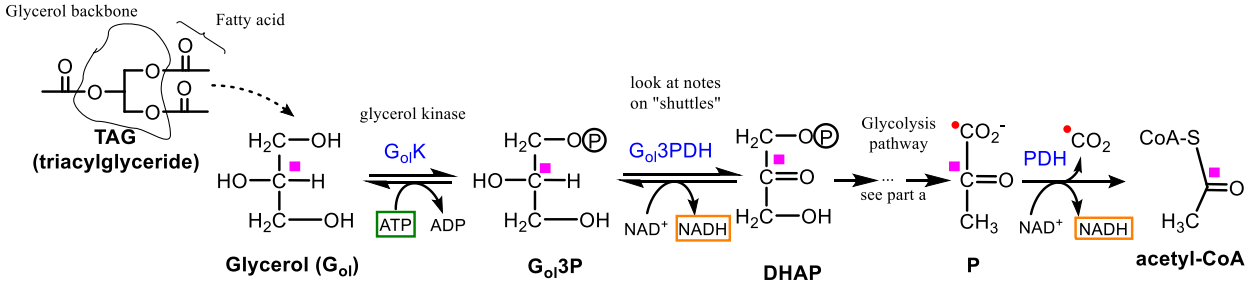


So, the carbons in glycogen lost as CO_2 at the PDH step are the carbons C3 and C4 of each glucose unit.

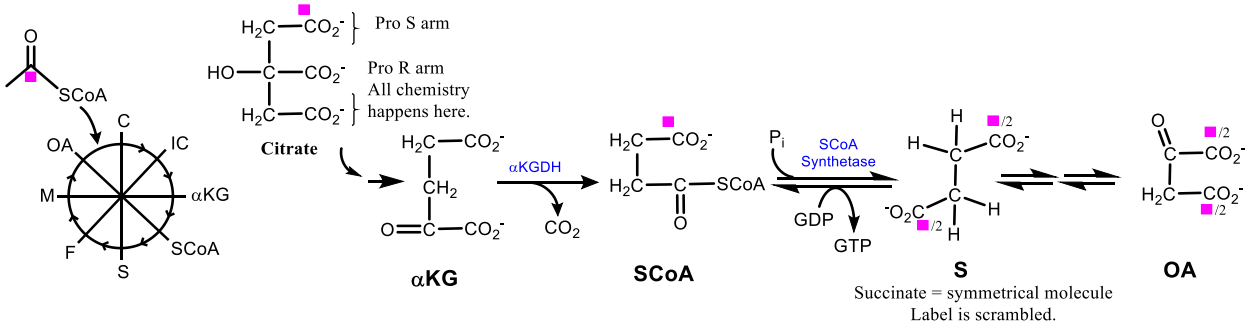


b. Glycerol is the backbone of triacylglycerides. The middle carbon is C2. Please trace a label from C2 (e.g., a ^{14}C or ^{13}C that has been inserted at that site by a synthetic reaction) through glycolysis and indicate the first place where the labeled molecule escapes as CO_2. Again, draw the pathway in sufficient detail to show us that you know what you are doing.

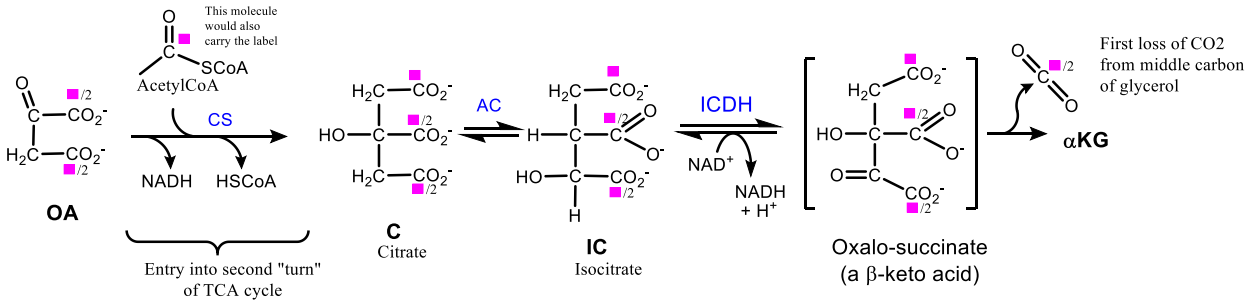
The C2 carbon of glycerol is marked with a purple square.



The acetyl-CoA enters the TCA cycle. Trace the labeled carbon around the TCA cycle – and notice that in the first pass, no labeled CO_2 is produced.



In the second turn of TCA cycle, however, labeled CO_2 (from the OA carboxyl group) escapes at the ICDH step.

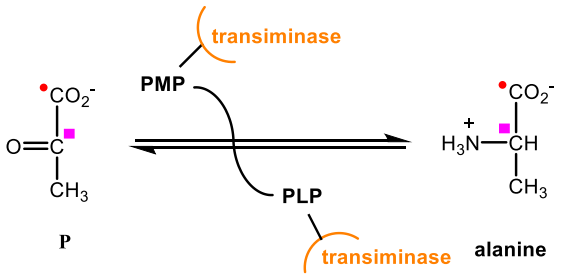


c. In Part b), there may be an energy yield between entry of glycerol into catabolism and the first exit of CO_2 from glycerol metabolism. On your pathway of Part b), count consumed and generated NAD+/NADH, FAD/FADH2, ADP and ATP, etc. and let us know the value of this catabolic conversion. Consider one NADH to be worth 3 ATP and one $FADH_2$ to be worth 2 ATPs.

Energy Balance on Glycerol Metabolism	ATP Equivalents
(i) Glycerol kinase : (-1) ATP	-1
(ii) $G_{ol}3$ P DH : +1 NADH	3
(iii) DHAP ⇌ GAP → (+ P_i, − NAD^+ → NADH) 1,3BPG : +1 NADH	3
(iv) PGlyK : +1 ATP	1
(v) PK : +1 ATP	1
(vi) PDH : +1 NADH	3
(vii) TCA : +3 NADH, +1 $FADH_2$, +1 GTP	12
TOTAL:	~22 ATP

Note that only the first pass of the TCA cycle is included in the above calculation, because by that point, exactly three carbons have been lost as CO_2, corresponding to the three carbons of glycerol. This gives us the net yield of ATP from one molecule of glycerol.

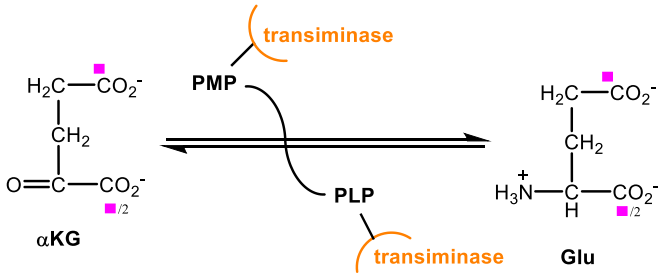
d. Look at your pathway in Part a. Trace the labeled carbon not to CO_2 (as before) but to alanine. Indicate any cofactor or energy needs for this transformation.



The C3 or C4 carbons in glycogen (from part a, red dots) end up as the carboxyl group in alanine. The C2 carbon of glycerol (from part b, purple square) ends up as the alpha carbon in alanine. This is a transamination reaction and requires

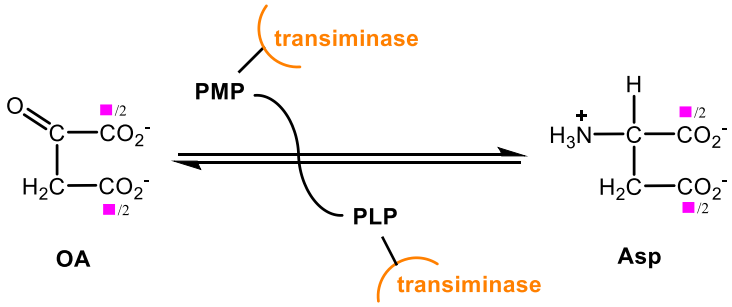
pyridoxal phosphate (PLP) as a cofactor. In this case, going from left to right, it requires the aminated form of PLP, which is pyridoxamine (PMP). See class notes and other materials for details on PLP-catalyzed transamination.

e. Look at your pathway of Part b. Trace the labeled carbon to the amino acid, glutamate. Indicate any cofactor or energy needs for this transformation.



Similar to part d, this is a PLP-catalyzed transamination reaction.

f. Look once again at your pathway of Part b. Trace the labeled carbon to the amino acid, aspartate. Indicate any cofactor or energy needs for this transformation.



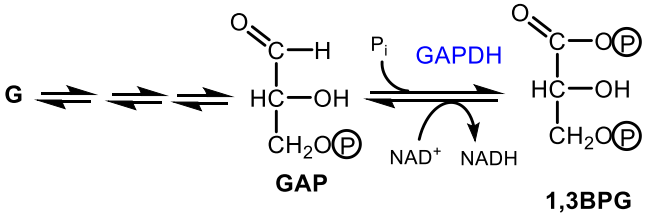
Similar to parts d and e, this is a PLP-catalyzed transamination reaction.

Problem 2

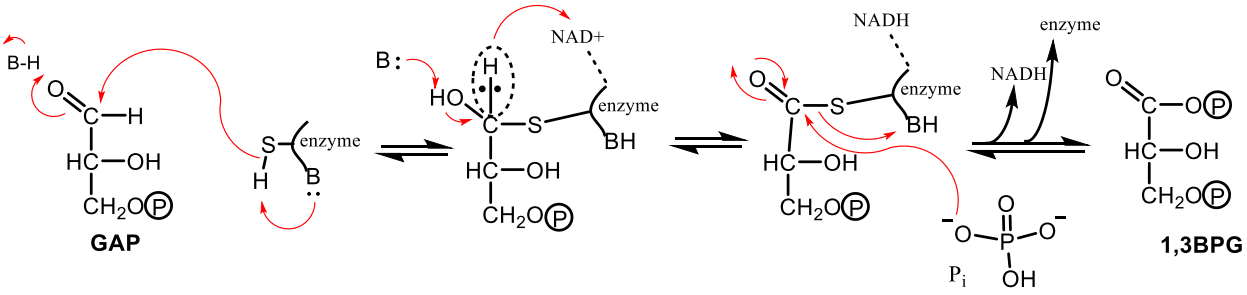
Heavy metal toxicology.

a. In ancient Rome, it was fashionable for men and women to have a pale complexion (e.g., a sun tan was not socially acceptable). A woman named Toffana made cosmetics out of arsenate (AsO_4^{3-}), which gave the desired effect (it caused anemia). Based on what JoAnne Stubbe taught in class, draw the step in which arsenate primarily blocks catabolism. Please draw the reaction as it is supposed to occur, and how it occurs in the presence of this toxic metal. Based upon what you know about the structures (including compartments) of red blood cells circulating in the blood vessels of people using these cosmetics, come up with an hypothesis to explain why these people developed anemia (giving them the desired cosmetic effect). Eventually hundreds of people died (including Toffana) so do not try this at home.

Arsenate inhibits the GAPDH reaction in glycolysis.

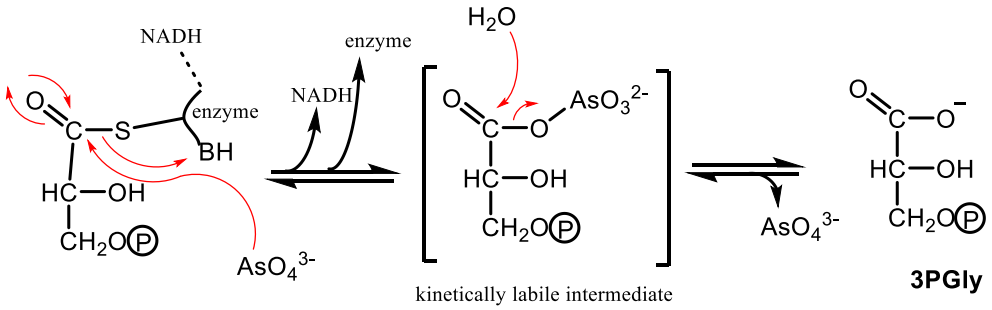


Normal GAPDH reaction:



The resulting 1,3BPG is kinetically stable, but thermodynamically labile; it gives off energy upon hydrolysis, and it helps generate ATP in the next step (PGlyKinase step).

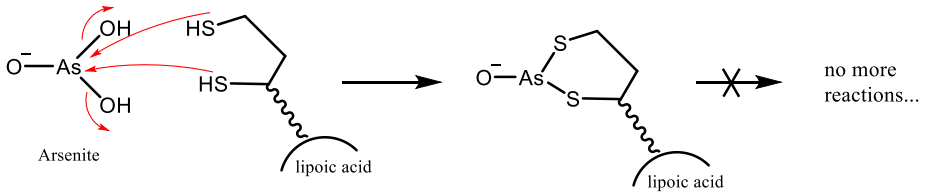
AsO_4^{3-} is a phosphate mimic; it reacts in place of P_i with the thioester, but the resulting arsenate ester hydrolyzes as fast as it forms.



You get no ATP from the PGlyKinase step, so no net ATP from glycolysis. Red blood cells lack mitochondria, so they cannot do respiration. Because they rely entirely on glycolysis, in the presence of arsenate, they "die"; hence you get anemia.

b. *Treponema pallidum* is the causative organism of syphilis. In the days before antibiotics, an early treatment was arsenite (AsO_3^{3-}), which has the property of bonding with closely spaced sulfhydryl groups. Based on what you know about the details of the pathways you have studied so far, predict the step at which arsenite would be active. Do you think it would cause anemia, as did its cousin, arsenate?

Arsenite reacts with "closely spaced" sulfhydryl – the places we have seen closely spaced sulfhydrils is in lipoic acid residues in PDH and αKGDH.

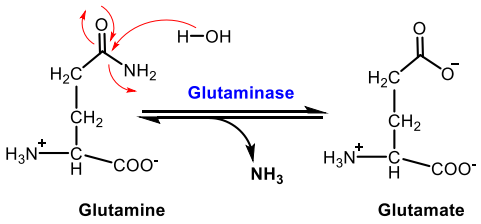


This compound "poisons" respiration. Apparently, *Treponema* is more sensitive than we are. However, unlike arsenate, arsenite has little effect on red blood cells, so it will not cause anemia.

Problem 3

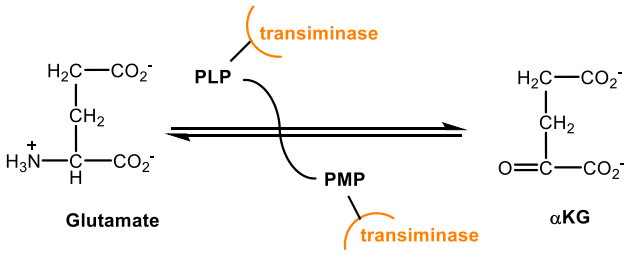
While we usually think of glucose or fatty acids as the primary precursors for catabolic energy generating pathways, mammalian cells especially like to use glutamine and glutamate, which are excellent carbon and nitrogen sources.

a. Predict in detail how glutamine might be converted enzymatically to glutamate.



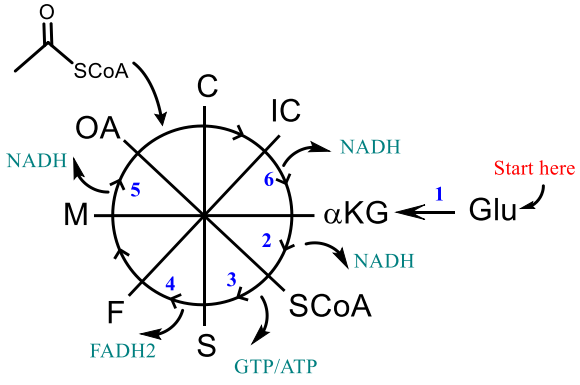
Details on the glutaminase reaction can be found in the book in the "Breakdown of amino acids" chapter (Voet and Voet, 3rd ed., page 751).

b. Predict, again showing structural details, how glutamate could be converted into a precursor for the TCA cycle, which is one of the catabolic pathways we have studied.



This is the same reaction from Problem 1, part e, but shown here in reverse.

c. Follow one molecule of glutamate around one turn of the TCA cycle and let us know how much energy is yielded (in terms of the amount of ATP, GTP that could be made). As above, assume one NADH converts to 3 ATP and one FADH2 converts to two ATP equivalents.



Step	ATP Equivalents
1	0
2	3
3	1
4	2
5	3
6	3
	12 ATP for one round of TCA cycle

Problem 4

Following is a list of hereditary or induced metabolic defects involving loss of single enzymes of catabolism, and a second list of possible consequences of such defects. Match each enzyme with its most likely consequence (only one) from the second list. Explain each answer by using, if appropriate, an abbreviated metabolic pathway.

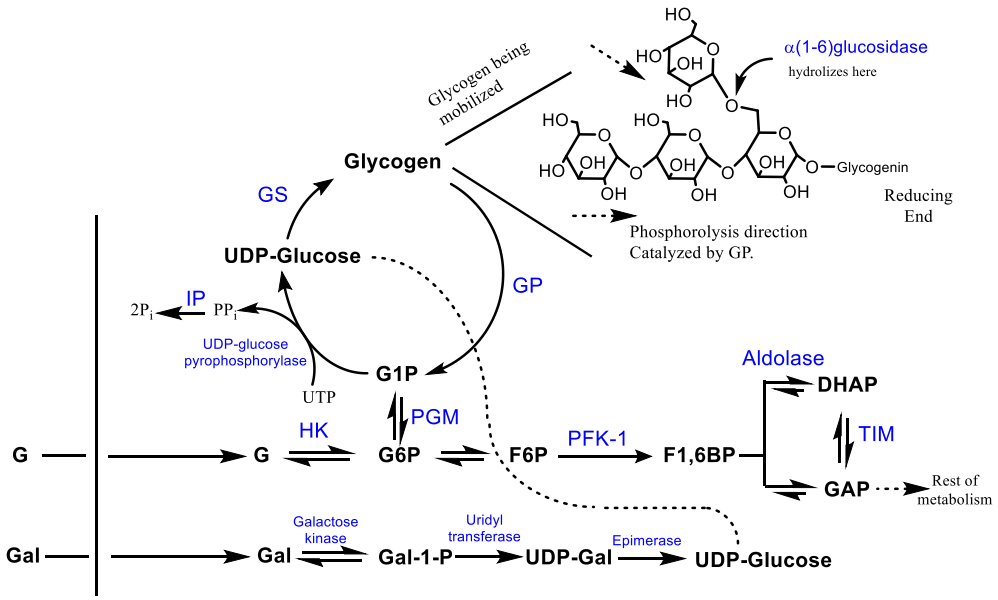
Defects:

a. Lack of phosphoglucomutase (PGM)
b. Lack of UDP-glucose pyrophosphorylase (not studied yet so you need to look it up)
c. Lack of triose phosphate isomerase (TPI or TIM)
d. Lack of phosphofructokinse I (PFK-I)
e. Lack of alpha-1,6-glucosidase (read about glycogen structure)

Consequences:

1. Lower than normal production of glucose 1-P in response to a sudden, large increase in cAMP level (cAMP is part of the "signal" mentioned in John's first lecture)
2. Lethal; prevents use of carbohydrates for ATP production
3. Inability to make glycogen unless galactose is available, with no effect on ability to use glycogen
4. Impaired ability to obtain energy from carbohydrates
5. Inability to use either glycogen or galactose as an energy source

Abbreviated metabolic pathway:



a. Lack of phosphoglucomutase (PGM) – the best match is 5. Carbon enters glycolysis from both galactose and glycogen by way of G1P (look back at Problem Set 5 for the epimerization mechanism by which Gal-1-P is converted to G1P). A deficiency in PGM would make it impossible to utilize either glycogen or galactose as an energy source. Note that carbon from galactose bypasses the PGM step and indeed could become part of the glycogen pool.

b. Lack of UDP-glucose pyrophosphorylase (not studied yet so you need to look it up) – the best match is 3. I wanted to show is that you cannot make glycogen from glucose if you lack UDP-glucose pyrophosphorylase (see diagram). However, galactose bypasses this step and, indeed, can be used to make glycogen. Once available, glycogen can be used in metabolism to generate energy.

c. Lack of triose phosphate isomerase (TPI or TIM) – the best answer is 4. The products of aldolase are DHAP and GAP. If they do not interconvert via TIM, the energy that otherwise would be gained from DHAP is lost.

d. Lack of phosphofructokinse I (PFK-I) – the best match is 2. If you cannot phosphorylate F6P, you cannot catabolize carbohydrates. This would be a lethal mutation. (The Doberman Pincer would catch you.)

e. Lack of alpha-1,6-glucosidase (read about glycogen structure) – the best answer is 1. The cAMP response to stress, hunger, etc., signals the need to break down glycogen to produce glucose and glucose phosphates. The lack of a de-branching enzyme would limit the energy precursors that could be made available for glycolysis (and gluconeogenesis, which we shall cover later in 5.07).

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