






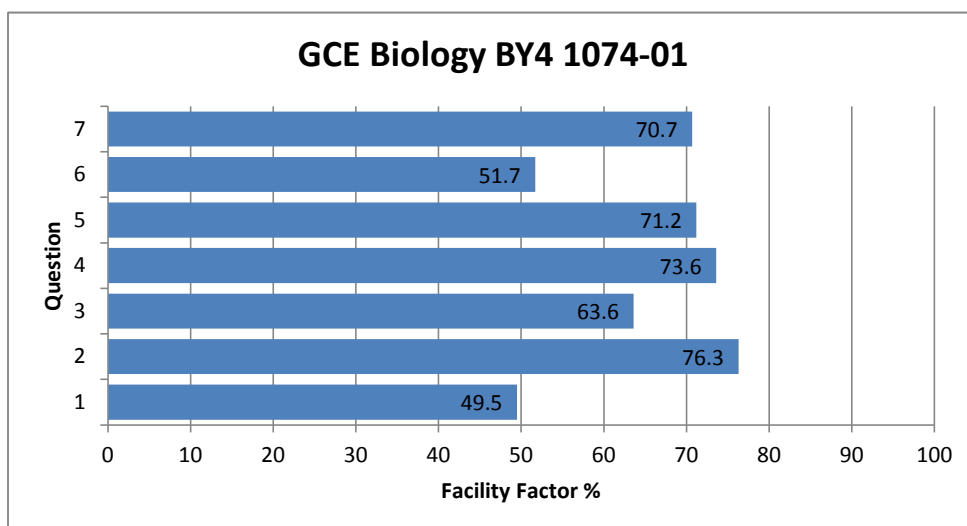


## GCE Biology BY4 1074-01

All Candidates' performance across questions

						
Question Title	N	Mean	S D	Max Mark	FF	Attempt %
1	3974	3	1.3	6	49.5	99.9
2	3974	5.3	1.5	7	76.3	99.9
3	3976	8.9	2.5	14	63.6	100
4	3974	10.3	2.9	14	73.6	99.9
5	3974	9.3	2.5	13	71.2	99.9
6	3974	8.3	3.1	16	51.7	99.9
7	3970	7.1	2.4	10	70.7	99.8



*Answer all questions.*

1. **Nitrogenases** are enzymes used by some organisms to fix atmospheric nitrogen gas ( $N_2$ ) into a form of nitrogen available to plants.

The **nif gene** is the gene coding for the synthesis of nitrogenases, found in nitrogen fixing bacteria.

*Rhizobium* is a mutualistic nitrogen fixing bacteria forming a relationship with legume species. In some species of *Rhizobium*, the nif genes are located on plasmids.

- (a) Name the *form* of nitrogen produced by *Rhizobium* that is '*available to plants*'. [1]

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- (b) Name *another* genus of nitrogen fixing bacteria. [1]

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- (c) State *precisely* where *Rhizobium* would be found in the legume. [1]

.....

- (d) Suggest how the relationship between *Rhizobium* and a legume species is beneficial to **both** organisms. [2]

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- (e) What are *plasmids*? [1]

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Rhizobium within the root nodules

allow plants to take up nitrates from the soil and the plant provides the Rhizobium with nutrients.

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A molecule that can carry genes.


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small, ~~rand~~ circular pieces of DNA that code for primary protein structure.


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2. (a) Define the following terms with reference to bacterial growth.

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(i) obligate aerobe

.....

.....

(ii) obligate anaerobe

.....

.....

(iii) facultative anaerobe

.....

.....

(b) Describe and explain the appearance of Gram positive and Gram negative bacteria following Gram staining.

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(c) Most pathogens in humans are Gram-positive organisms. Six Gram-positive genera are typically pathogenic in humans. Two of these, *Streptococcus* and *Staphylococcus*, are cocci. The remaining organisms are bacilli.

What **three dimensional shape** would the cocci and bacilli be?

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cocci .....

bacilli .....

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The stain, crystal violet, is added and all Gram positive bacteria retain the dye so turn purple, then a counterstain, safranin, is added and Gram negative bacteria turn red. Gram negative bacteria do not retain crystal violet dye because they have more \*

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cocci spherical shaped

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An obligate aerobe bacteria is a bacteria which can only survive in the presence of oxygen.

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An obligate anaerobe bacteria is a bacteria which can only survive in the absence of oxygen, if oxygen is not present.

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A facultative anaerobe bacteria is a bacteria which can survive in the absence of oxygen but lives/survives better with oxygen present.

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Gram positive bacteria would be stained violet because the thick peptidoglycan layer <sup>retains</sup> the violet stain.

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Ideally these bacteria respire best in aerobic (oxygenated) conditions, so grow best with oxygen. However when no oxygen is present they are also able to respire anaerobically so can still grow.

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Gram positive bacteria are purple after gram staining as they ~~are~~ have a thick peptidoglycan cell wall <sup>trap</sup> with crosslinks that absorb and retain the dye. Gram negative are red, as they have a thin <sup>peptidoglycan</sup> layer with a thick outer layer of lipopolysaccharide. The purple dye is easily washed away so they go red in the safranin counterstain.

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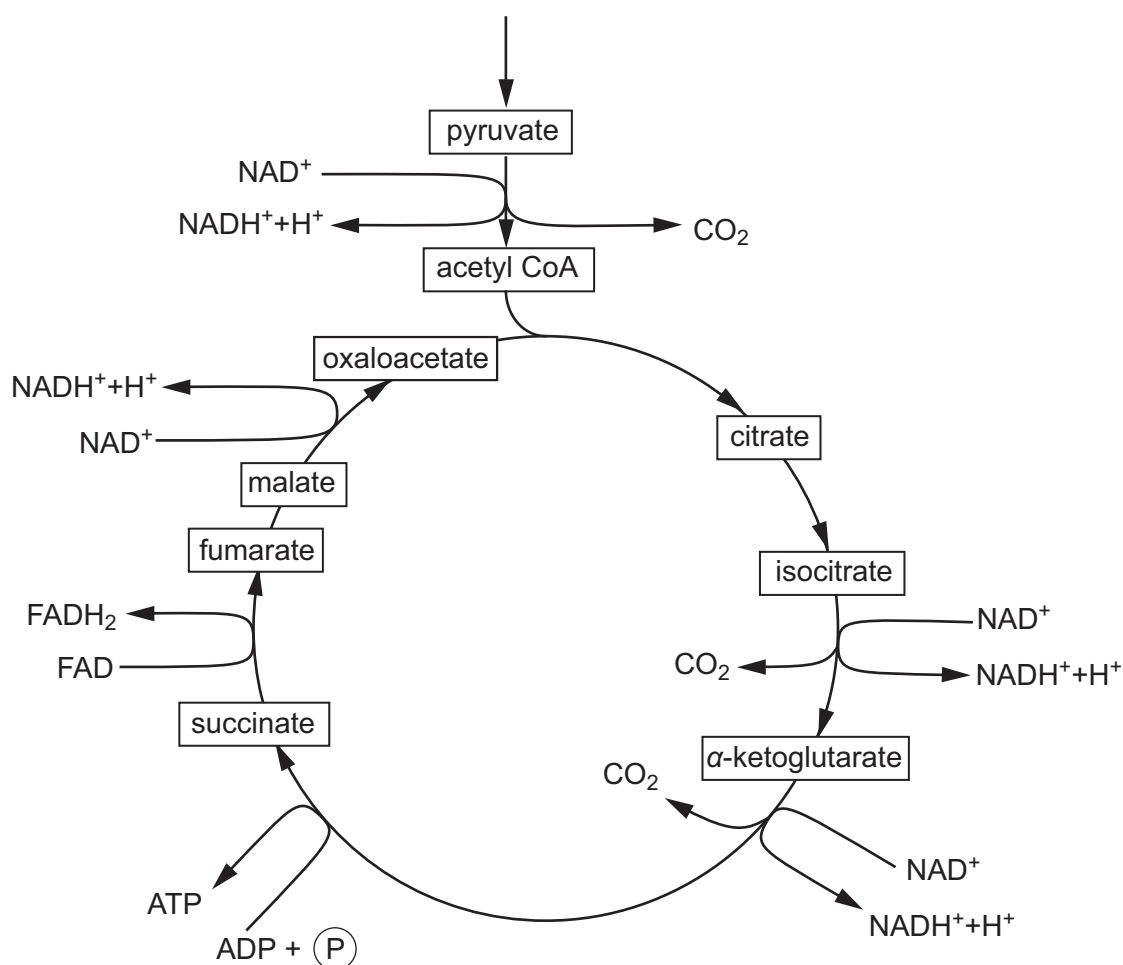
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6. The detailed diagram below shows the link reaction and Krebs cycle. Most of the intermediates involved are named.



- (a) (i) Using the diagram above, and your own knowledge, determine how many carbons there are in the following Krebs cycle intermediates. [1]

citrate .....

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succinate .....

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- (b) Briefly describe how reduced FAD and reduced NAD are used to create an electrochemical gradient. [4]

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In patients with a suspected mitochondrial disorder a minimally invasive tissue biopsy provides the best opportunity to examine mitochondrial function, freezing the sample immediately after collection.

In addition to activity measurements of individual enzymes, analysis of mitochondrial respiration and ATP production rates are performed. This includes the measurement of mitochondrial oxygen consumption in the presence of different substrates, such as pyruvate and  $\alpha$ -ketoglutarate. Analysis may show increased levels of a Krebs cycle intermediate, such as malate and succinate.

Defects in mitochondrial ATP synthesis may lead to high lactate levels in blood.

*J Inherit Metab Dis. 2011 April; 34(2): 283–292.*

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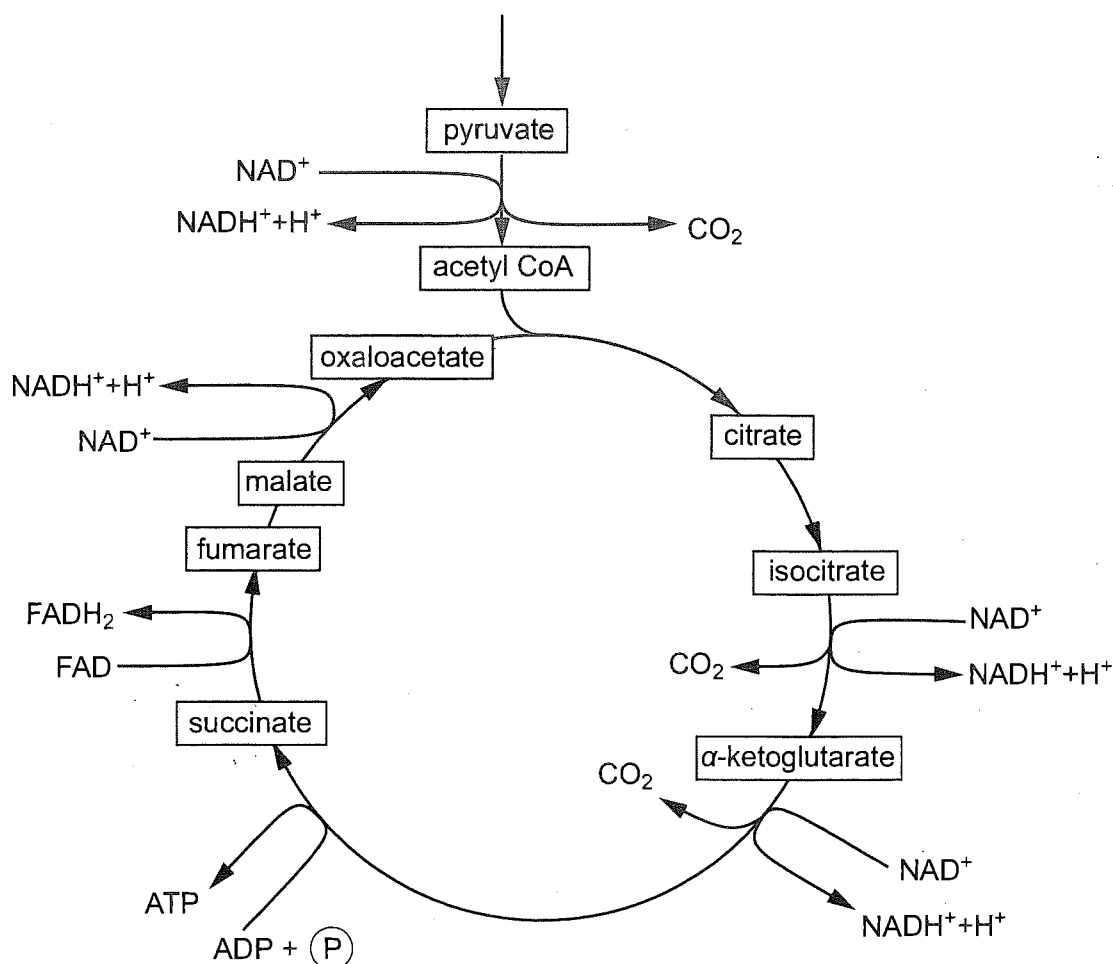
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the acetyl which is added ~~at~~ bonded to the oxaloacetate to form citrate contains 2 carbons. At each removal of  $\text{CO}_2$  a carbon is lost,  $\therefore$  the next intermediate will have 1 less carbon.

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In the electron transport chain,  $\text{NADH}_2$  and  $\text{FADH}_2$  donate hydrogen ions and electrons. As the electron or at proton pumps. As electron are moved between carrier, hydrogen ions are pumped into the intermembrane space. The membrane is impermeable to ions, so there is an accumulation of hydrogen ions creating a electrochemical gradient.

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muscle, contains high amounts of mitochondria.

- (e) What could be deduced if the oxygen consumption was low with the pyruvate as a substrate but high with  $\alpha$ -ketoglutarate as a substrate? [2]

That the conversion of pyruvate does not require ATP but the conversion of  $\alpha$ -ketoglutarate does.

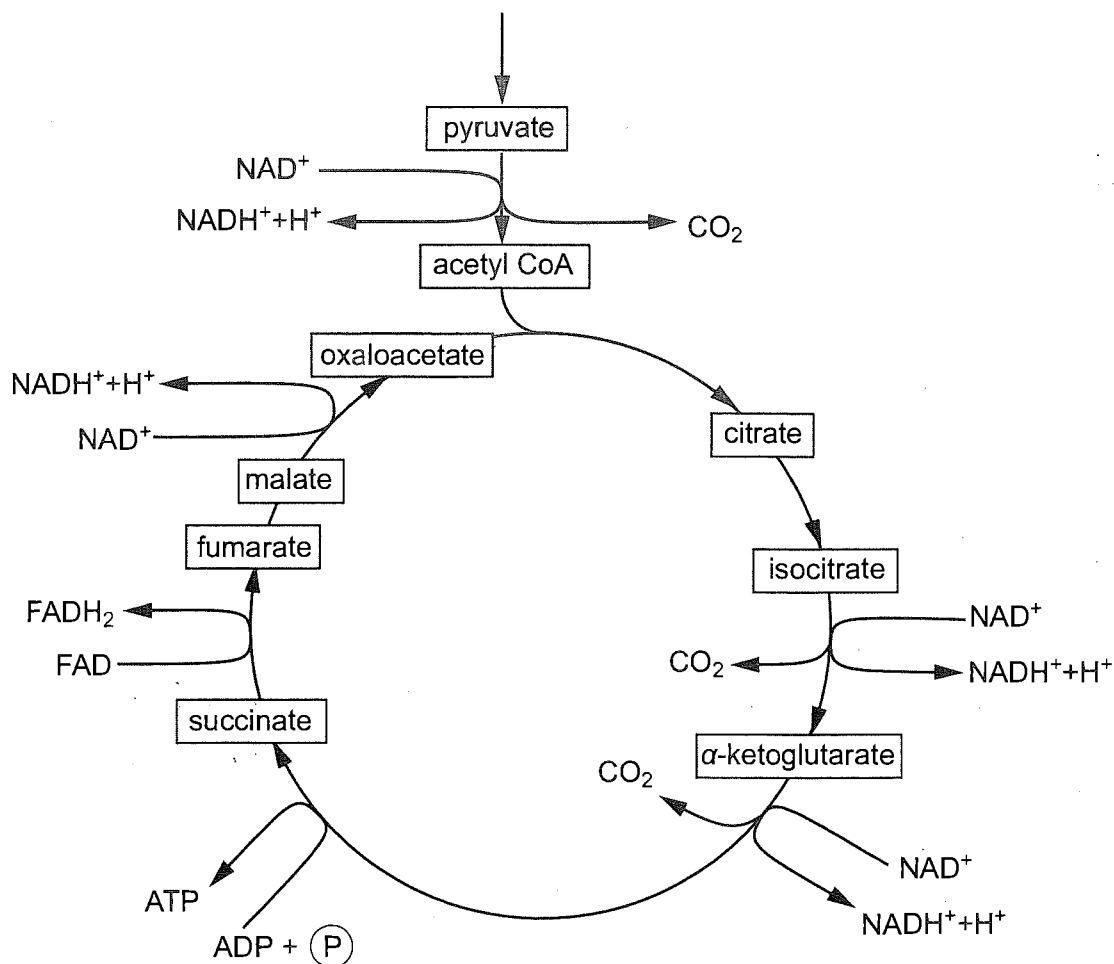
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~~There is a shortage of NAD and FAD.~~  
That the mitochondria are not functioning.

- (g) Explain why there is a raised blood lactate level in many patients with mitochondrial disease. [2]

There is not enough ~~enough~~ ATP for aerobic respiration to occur so anaerobic respiration takes place producing lactic acid.

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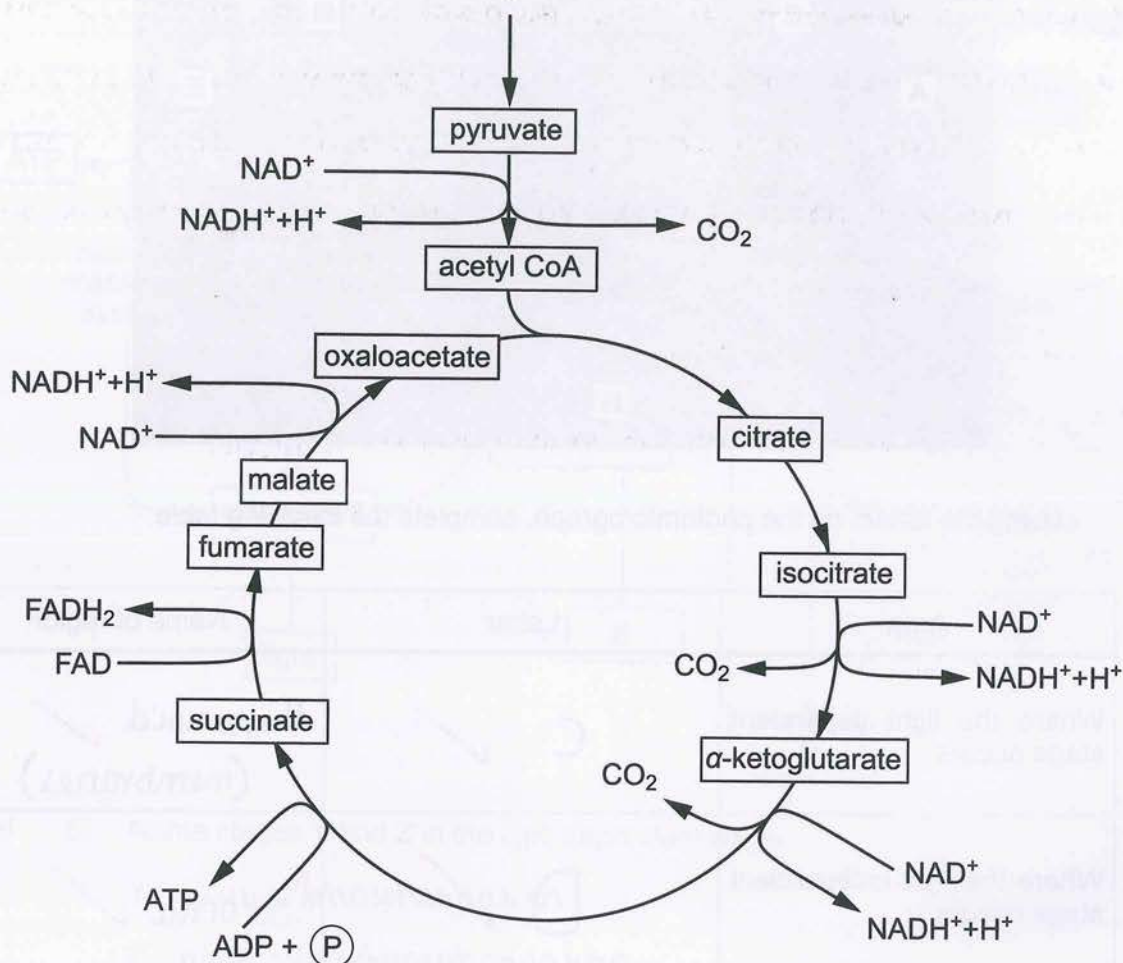
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Examiner  
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Citrate is produced from combining <sup>oxa</sup>lacetate, a four carbon compound, with acetyl coenzyme A, a two carbon compound, and is thus a six carbon compound.  $\alpha$ -ketoglutarate is produced following the decarboxylation of isocitrate, where ~~CO<sub>2</sub> is removed~~

\* CONTINUED IN ADDITIONAL BOOKLET

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Reduced FAD and reduced NAD donate protons and electrons to the electron transport chain. The electrons pass along a series of electron carriers at increasingly lower energy levels. This releases energy which is used to fuel proton pumps. Proton pumps pump protons across the inner mitochondrial membrane from the matrix into the inter-membrane space. Protons accumulate in the inter-membrane space and thus an electrochemical

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A suitable tissue would be the leg muscle because there are many mitochondria here to produce ATP for muscle contraction and it is safe for the patient because the muscle is easily accessible and easily healed.



- (e) What could be deduced if the oxygen consumption was low with the pyruvate as a substrate but high with  $\alpha$ -ketoglutarate as a substrate? [2]

This would suggest that ~~glycolysis~~ <sup>the link reaction</sup> was not ~~working~~ <sup>functioning</sup> but that the krebs cycle was ~~working~~ <sup>functioning</sup>. Oxygen consumption is proportional to rate of respiration because oxygen is the final electron acceptor in the electron transport chain. If oxygen consumption is low with \*

- (f) What could be deduced if there was a build up of any one of the Krebs cycle intermediates? [1]

That there is a problem in breaking down these krebs cycle intermediates perhaps the enzymes that usually do this cannot function properly.

- (g) Explain why there is a raised blood lactate level in many patients with mitochondrial disease. [2]

Because aerobic respiration does not take place as efficiently, so patients have to rely on anaerobic respiration to <sup>produce</sup> ~~gain~~ ATP. Anaerobic respiration produces lactate.

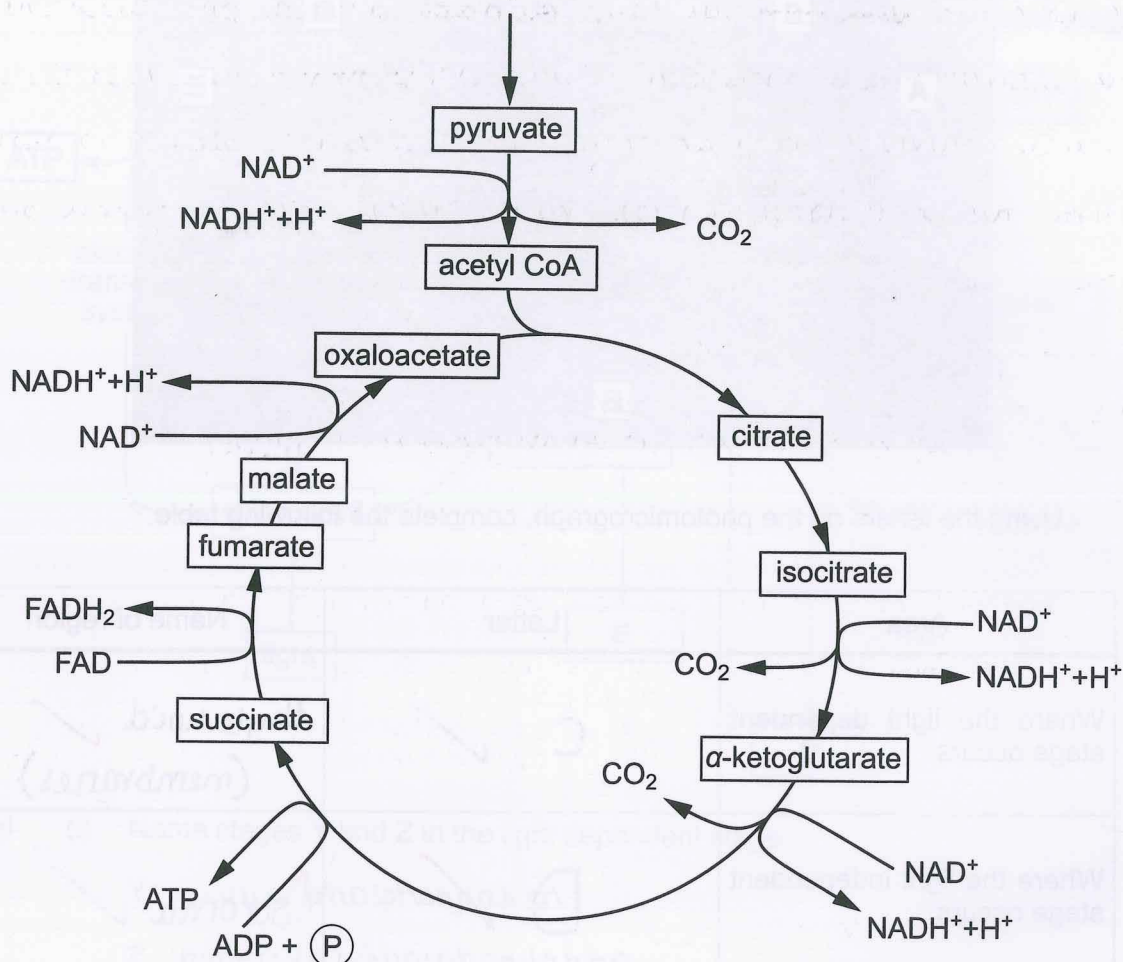
6a11 one molecule of carbon dioxide is removed, leaving a five carbon compound. Succinate is produced following the decarboxylation of  $\alpha$ -ketoglutarate and is thus a four carbon compound (since it contains one less carbon).

6b gradient is created. ~~FAD only releases its results in two~~ With FAD, electrons pass along <sup>two</sup> ~~three~~ electron carriers whereas with NAD, electrons pass along three electron carriers.

6e pyruvate, this would suggest that pyruvate cannot be broken down as efficiently. Since it is broken down using the same enzymes that are involved in the Krebs cycle, this perhaps suggests that there is insufficient <sup>quantities of</sup> coenzyme A to bond with acetate. However, since there is ~~is~~ high oxygen consumption with  $\alpha$ -ketoglutarate, this suggests that ~~it~~ ~~is~~ ~~the~~



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Citrate is produced from combining <sup>oxa</sup>lloacetate, a four carbon compound, with acetylcoenzyme A, a two carbon compound, and is thus a six carbon compound.  $\alpha$ -ketoglutarate is produced following the decarboxylation of isocitrate, where ~~CO<sub>2</sub>~~ is removed.

\* CONTINUED IN ADDITIONAL BOOKLET

- (b) Briefly describe how reduced FAD and reduced NAD are used to create an electrochemical gradient. [4]

Reduced FAD and reduced NAD donate protons and electrons to the electron transport chain. The electrons pass along a series of electron carriers at increasingly lower energy levels. This releases energy which is used to fuel proton pumps. Proton pumps pump protons across the inner mitochondrial membrane from the matrix into the inter-membrane space. Protons accumulate in the inter-membrane space and thus an electrochemical

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In patients with a suspected mitochondrial disorder a minimally invasive tissue biopsy provides the best opportunity to examine mitochondrial function, freezing the sample immediately after collection.

In addition to activity measurements of individual enzymes, analysis of mitochondrial respiration and ATP production rates are performed. This includes the measurement of mitochondrial oxygen consumption in the presence of different substrates, such as pyruvate and  $\alpha$ -ketoglutarate. Analysis may show increased levels of a Krebs cycle intermediate, such as malate and succinate.

Defects in mitochondrial ATP synthesis may lead to high lactate levels in blood.

*J Inherit Metab Dis. 2011 April; 34(2): 283–292.*

- (c) Name the **two** enzyme **types** involved in the conversion of pyruvate to acetyl CoA. [2]

decarboxylase

dehydrogenase

Using the diagram opposite, the text above and your own knowledge, answer the following questions.

- (d) Suggest a suitable tissue to examine mitochondrial function and explain why you have chosen this tissue with respect to patient safety. [2]

A suitable tissue would be the leg muscle because there are many mitochondria here to produce ATP for muscle contraction and it is safe for the patient because the muscle is easily accessible and easily healed.



- (e) What could be deduced if the oxygen consumption was low with the pyruvate as a substrate but high with  $\alpha$ -ketoglutarate as a substrate? [2]

This would suggest that ~~glycolysis~~ <sup>the link reaction</sup> was not ~~working~~ <sup>functioning</sup> but that the krebs cycle was ~~working~~ <sup>functioning</sup>. Oxygen consumption is proportional to rate of respiration because oxygen is the final electron acceptor in the electron transport chain. If oxygen consumption <sup>low with \*</sup>

- (f) What could be deduced if there was a build up of any one of the Krebs cycle intermediates? [1]

That there is a problem in breaking down these krebs cycle intermediates perhaps the enzymes that usually do this cannot function properly.

- (g) Explain why there is a raised blood lactate level in many patients with mitochondrial disease. [2]

Because aerobic respiration does not take place as efficiently, so patients have to rely on anaerobic respiration to <sup>produce</sup> gain ATP. Anaerobic respiration produces lactate.



6a ii one molecule of carbon dioxide is removed, leaving a five carbon compound. Succinate is produced following the decarboxylation of  $\alpha$ -ketoglutarate and is thus a four carbon compound (since it contains one less carbon).

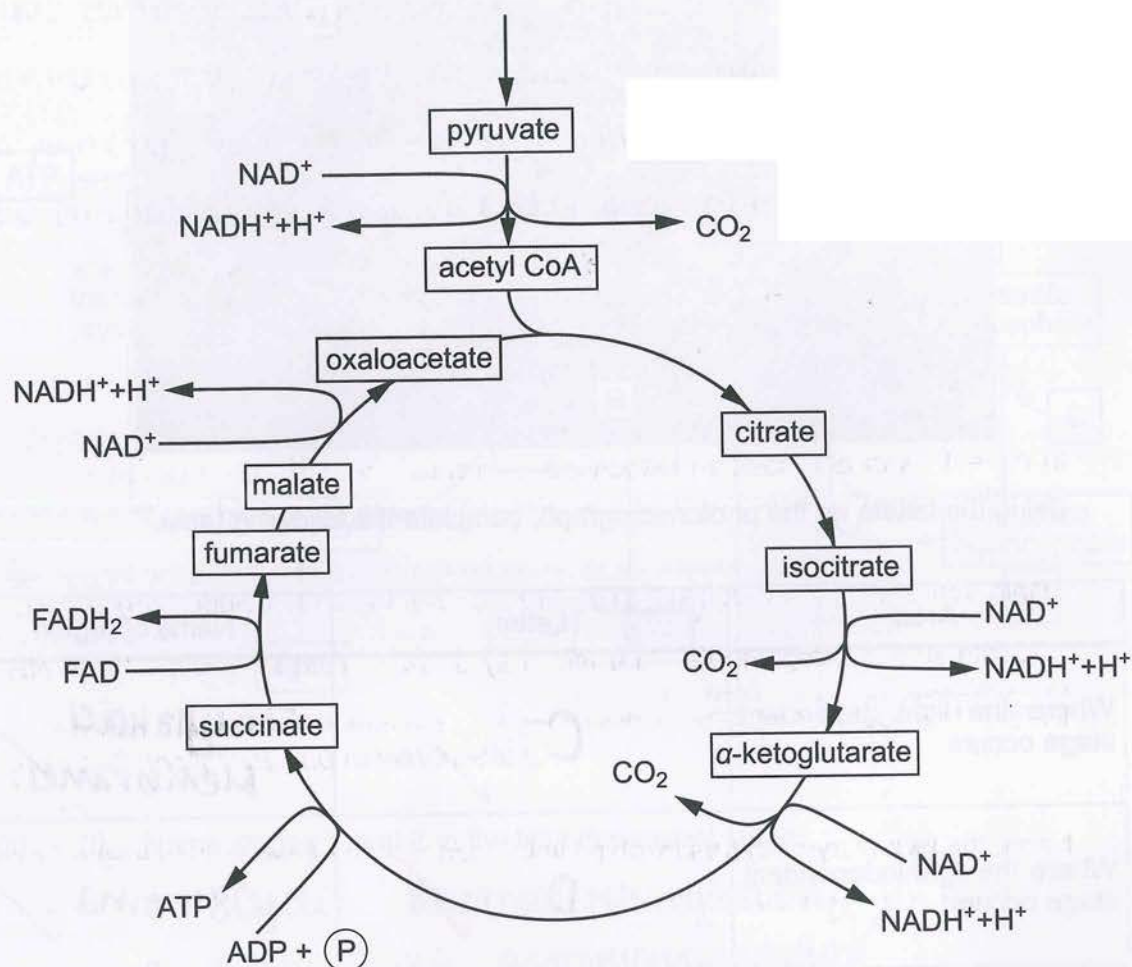
6b gradient is created. ~~FAD only releases its results in two~~ With FAD, electrons pass along ~~three~~ <sup>two</sup> electron carriers whereas with NAD, electrons pass along three electron carriers.

6c pyruvate, this would suggest that pyruvate cannot be broken down as efficiently. Since it is broken down using the same ~~enzymes~~ that are involved in the Krebs cycle, this perhaps suggests that there is insufficient <sup>quantities of</sup> coenzyme A to bond with acetate. However, since ~~more of~~ <sup>high</sup> oxygen consumption with  $\alpha$ -ketoglutarate, this suggests that ~~it is~~





6. The detailed diagram below shows the link reaction and Krebs cycle. Most of the intermediates involved are named.



- (a) (i) Using the diagram above, and your own knowledge, determine how many carbons there are in the following Krebs cycle intermediates. [1]

citrate ..... 6 .....

$\alpha$ -ketoglutarate ..... 5 .....

succinate ..... 4 .....

- (ii) Explain precisely how you have arrived at these figures. [2]

A citrate is 2 carbon, and oxaloacetate is 4 so they combine to give 6 carbon citrate. It goes through 1 decarboxylation where  $\text{CO}_2$ , hence 1 carbon, is removed to form  $\alpha$  ketoglutarate, hence 5. Another decarboxylation where  $\text{CO}_2$ , hence 1 carbon, is removed occurs to give succinate, which is four carbon.



- (b) Briefly describe how reduced FAD and reduced NAD are used to create an electrochemical gradient. [4]

NADH and FAD, when reduced, move to the electron transport chain. Here the hydrogen dissociates into  $H^+$  ions (protons) and electrons. The electrons travel down a chain of electron carriers, and the energy lost by them provides energy for proton pumps to pump protons from the matrix, into the intermembrane space across the inner membrane. The membrane is impermeable to protons flowing back, so a high concentration of  $H^+$  ions (protons) is built up  
[continued in separate booklet]

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Decarboxylase

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Muscle tissue. This is easy to obtain without anaesthetic, and contains a lot of mitochondria per unit of space so only a small amount would be needed to analyse. It is easy to do this aseptically so infection risk would be minimised. The ideal place of removal is the thigh which has a high level of muscle and will not harm any vital organs, so the patient is as safe as possible.



- (e) What could be deduced if the oxygen consumption was low with the pyruvate as a substrate but high with  $\alpha$ -ketoglutarate as a substrate? [2]

This could indicate that the disease is preventing the link reaction <sup>that convert pyruvate</sup> from occurring, possibly by inhibiting the specific enzyme, or stopping pyruvate entering the mitochondrion. This would prevent the production of a lot of  $\text{NADH}_2$  and  $\text{FADH}_2$ , meaning that oxygen would not be used so much as the final electron acceptor.

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It could be deduced that the specific dehydrogenase or decarboxylase enzyme that acts on that intermediate is inhibited or not synthesised due to the disease.

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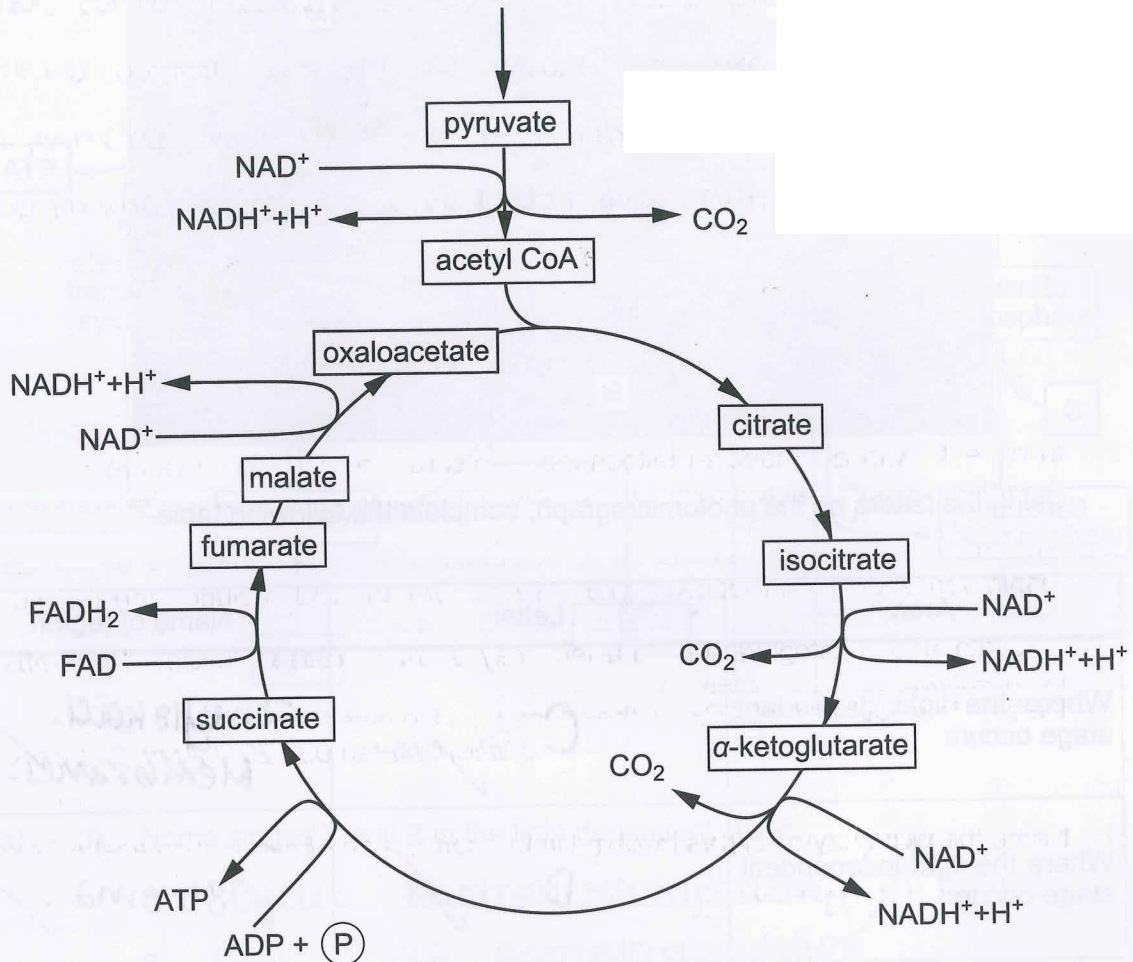
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