



Oxford Cambridge and RSA

Friday 16 October 2020 – Morning

A Level Biology B (Advancing Biology)

H422/02 Scientific literacy in biology

Time allowed: 2 hours 15 minutes

You must have:

- the Insert
- a clean copy of the Advance Notice (inside this document)

You can use:

- a scientific or graphical calculator
- a ruler (cm/mm)



Please write clearly in black ink. **Do not write in the barcodes.**

Centre number

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Candidate number

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First name(s)

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Last name

StemUp Model Solution

INSTRUCTIONS

- Use black ink. You may use an HB pencil for graphs and diagrams.
- Write your answer to each question in the space provided. If you need extra space use the lined pages at the end of this booklet. The question numbers must be clearly shown.
- Answer **all** the questions.
- Where appropriate, your answer should be supported with working. Marks might be given for using a correct method, even if your answer is wrong.

INFORMATION

- The total mark for this paper is **100**.
- The marks for each question are shown in brackets [].
- Quality of extended response will be assessed in questions marked with an asterisk (*).
- This document has **20** pages.

ADVICE

- Read each question carefully before you start your answer.

Answer **all** the questions.

- 1 This question is based on the Advance Notice article ‘**Mitochondrial DNA and Mitochondrial Disease**’, on the **Insert**.

(a) Mitochondrial DNA (mtDNA) is much smaller than nuclear DNA and is similar to prokaryotic DNA.

(i) Give **two** ways, apart from size, in which mtDNA differs from nuclear DNA.

1. *mtDNA is circular, whereas nuclear DNA is linear.*

2. *mtDNA is not associated with histones (proteins) and contains no introns (little junk DNA).*

[2]

(ii) Complete the following table of comparisons between mtDNA, RNA and ATP. Put a tick (✓) in each box where that component is present.

The first line has been completed for you.

Component	mtDNA	RNA	ATP
adenine	✓	✓	✓
ribose		✓	✓
uracil		✓	
pyrimidine	✓	✓	

[3]

(iii) The mitochondrial genome contains 37 genes. Of these, only 13 code for proteins.

Suggest what the remaining genes encode.

mitochondrial tRNA and rRNA (transfer RNA and ribosomal RNA)

[1]

(b) Like replication of nuclear DNA, replication of mtDNA is semi-conservative.

(i) Explain what is meant by the term semi-conservative replication.

Two identical DNA molecules are produced.
Each original strand is used as a template, so each new molecule contains one original (parental) strand and one newly synthesised strand: half the genetic information is kept in the new DNA.
[2]

(ii) Explain how mutations in the catalytic and exonuclease regions of the *POLG* gene could have different effects on the replication of mtDNA.

A mutation in the catalytic region could stop the polymerase working, so it prevents (slows) mtDNA replication.
A mutation in the exonuclease (proofreading) region reduces the accuracy of replication, so the mutation rate of mtDNA increases.
[2]

(c) Sequencing the mitochondrial genome has helped our understanding of the function of mitochondria and the basis of mitochondrial disease.

PCR is used to amplify the mtDNA in a sample before sequencing.

(i) In PCR, the DNA is placed in a buffered solution in a thermocycler.

State the **three** other components that are placed in the buffered solution in PCR.

1. (free, phosphorylated) nucleotides
2. Taq (thermostable) DNA polymerase
3. DNA primers

[3]

(ii) A sample contained 1500 molecules of mtDNA.

Calculate the number of mtDNA molecules you would expect after 15 cycles of PCR.

Express your answer as a $\log_{10}$ value.

$$\text{amplification factor} = 2^{15} = 32\,768$$

$$1500 \times 32\,768 = 49\,152\,000 \text{ molecules}$$

$$\log_{10} 49\,152\,000 = 7.69$$

number of molecules = ...7.69..... [2]

Turn over

- (iii) Place the events, **A** to **G**, in the correct order in the boxes below to describe the process of gel electrophoresis. The first one is done for you.

- A** DNA fragments are placed in wells by the cathode
- B** when the current is stopped, shorter fragments will finish nearer the anode
- C** UV light can be used to visualise banding patterns
- D** a current is applied
- E** the gel plate is covered in buffer solution
- F** DNA fragments can be extracted for further analysis
- G** the DNA fragments begin moving towards the anode

E	A	D	G	B	C	F
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[4]

- (d) The Advance Notice article describes the causes and symptoms of mitochondrial disease.

Explain why raised concentrations of lactate confirm diagnosis of mitochondrial disease.

If mitochondria are not functioning, there will be more anaerobic respiration.....
Functioning mitochondria are needed to re-oxidise reduced NAD (regenerate.....
NAD) via the electron transport chain, so instead pyruvate is converted to.....
lactate to regenerate NAD, and lactate accumulates.....

[2]

2 (a) Type 1 and type 2 diabetes have different treatments.

(i) Compare the treatments for type 1 and type 2 diabetes.

...Type 1 diabetes is treated with insulin injections (or an insulin pump),
 ...as the pancreas does not produce insulin.
 ...Type 2 diabetes is treated by a controlled diet with reduced intake of
 ...refined sugars, weight loss and increased exercise.
 ...If this is not enough, type 2 is treated with (named) drugs, e.g.
 ...metformin, and insulin only later if needed.

[3]

(ii) Explain why measurement of the concentration of glycosylated haemoglobin is used to evaluate how well a patient's blood glucose concentration has been controlled.

...The concentration of glycosylated haemoglobin (HbA1c) increases with
 ...blood glucose concentration, as glucose binds to haemoglobin.
 ...Red blood cells last about 120 days, so it indicates the average blood
 ...glucose concentration over several weeks.

[2]

- (b) Diabetes can lead to the development of a disease called retinopathy, which involves damage to the retina.

Some doctors have suggested that hypertension increases the risk of retinopathy in patients with type 1 diabetes.

A clinical trial was carried out to investigate the effect of blood pressure on the risk of developing retinopathy.

Two groups of patients with type 1 diabetes were selected and treated as follows:

- the test group of 231 patients received lisinopril, a drug that reduces hypertension
- the control group of 228 patients received a placebo

Both groups were monitored for a period of 24 months. Their blood pressure and glycosylated haemoglobin concentrations were measured regularly.

The results of the trial are shown in the table.

	Number of patients who developed retinopathy or whose retinopathy worsened	Number of patients whose retinopathy improved	Mean blood pressure at start of trial	Mean blood pressure at end of trial
Test group (lisinopril)	21	33	$\frac{123}{81}$	$\frac{120}{80}$
Control group (placebo)	39	28	$\frac{123}{81}$	$\frac{123}{81}$

- (i) Name an instrument used to measure blood pressure.

...sphygmomanometer..... [1]

- (ii) Calculate the percentage decrease in the systolic blood pressure in the test group over the course of the trial.

$$(123 - 120) \div 123 \times 100$$

$$= 3 \div 123 \times 100 = 2.439$$

percentage decrease = ...2.4..... % [2]

- (iii)* The doctors running the clinical trial concluded that lisinopril had beneficial effects on retinopathy caused by type 1 diabetes.

Evaluate this conclusion.

In your answer, suggest what additional information the doctors would need to be more confident in their conclusion.

The data partly support the conclusion. Fewer patients in the test group developed retinopathy or had it worsen (21) than in the control group (39), and more patients improved with lisinopril (33) than with the placebo (28). Systolic blood pressure decreased in the test group (123 to 120, a 2.4% decrease) but did not change in the control group. However, 21 patients on lisinopril still developed or worsened, and the difference in the number of improvements (33 v 28) is small. The decrease in blood pressure is only small, and blood pressure was in the normal range in both groups (no hypertension), so the effect may not be due to lowering hypertension. No HbA1c data were given, so glucose control may have differed between the groups. The sample size (about 230 per group) is relatively small and the trial lasted only 24 months. To be more confident the doctors need a larger trial and a statistical test to show the difference is significant (not due to chance). They [6]

Additional answer space if required.

need HbA1c data to correlate control of diabetes with retinopathy, information on the severity of diabetes in the two groups, whether the groups were age and sex matched, and whether patients took other medication or had pre-existing conditions.

- (c) One factor that contributes to damage to the retina is macular oedema, which is swelling in the eye.

Use your knowledge of tissue fluid formation to explain how hypertension could increase the incidence of oedema.

Hypertension increases the blood pressure, so hydrostatic pressure (HP) increases at the arterial end of the capillaries in the eye (retina). HP is then even greater than the oncotic pressure of the blood than normal, so more fluid is forced out of the capillaries. So more tissue fluid is formed than normal, and less tissue fluid returns to the capillaries, so it accumulates as oedema.

[3]

- (d) A researcher collected data for the incidence of hypertension and type 2 diabetes in a population.

- (i) Describe how the researcher could test for correlation between hypertension and type 2 diabetes.

Plot a scattergraph of the data, then calculate Spearman's rank correlation coefficient. Look up the critical value (p value) in a table to see if the correlation is significant at $p = 0.05$.

[2]

- (ii) The researcher found that there was a positive correlation between hypertension and type 2 diabetes.

As a result, they concluded that type 2 diabetes was caused by hypertension.

Explain why the researcher's conclusion may not have been correct.

Correlation does not imply causation. Both diseases may be caused by, or linked to, another factor, such as obesity (or poor diet, lack of exercise, age).

[2]

- 3 (a) Fig. 3.1 outlines the two stages of photosynthesis.

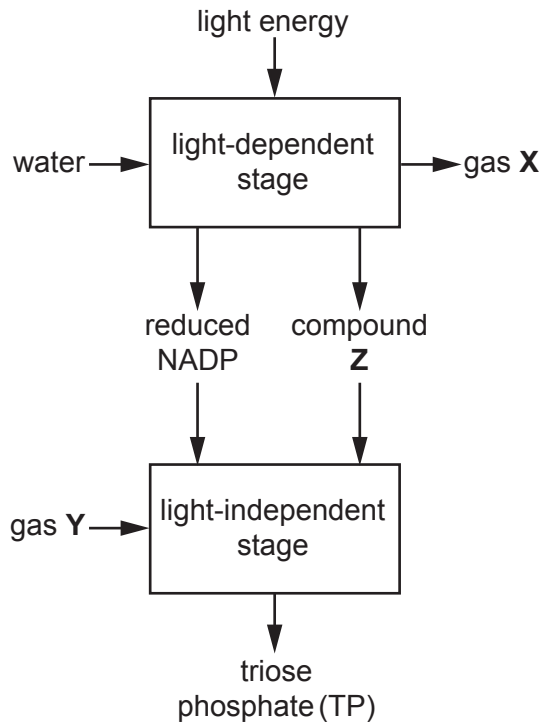


Fig. 3.1

- (i) Identify the following from Fig. 3.1:

gas X ..oxygen (O_2).....

gas Y ..carbon dioxide (CO_2).....

compound Z ..ATP (adenosine triphosphate).....

[2]

- (ii) Name the structure in the chloroplast in which the light-dependent stage of photosynthesis takes place.

..granum (grana, thylakoid membranes)..... [1]

- (iii) Describe **two** ways in which the structure you named in (ii) is adapted to its function.

..The thylakoid membranes contain photosynthetic pigments, e.g.

..chlorophyll a, in photosystems, and electron carriers (ETC) and ATP.....

..synthase.....

..Stacked thylakoids give a large surface area for light absorption and.....

..electron transport, and allow an electrochemical gradient to form..... [2]

- (b) A student used paper chromatography to analyse a mixture of photosynthetic pigments.

Fig. 3.2 shows the chromatogram produced. The student numbered each spot and marked the position of the centre of each spot with a pencil mark to help with measurement.

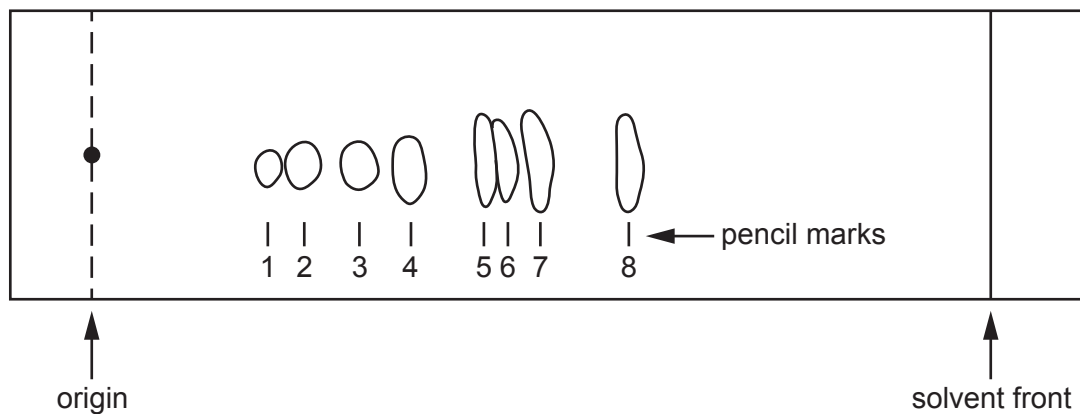


Fig. 3.2

- (i) Explain why the student did not allow the solvent to reach the end of the paper.

Otherwise the pigment spots would continue to move, and the solvent front would not be known, so R_f values would not be correct.

[1]

- (ii) Calculate the R_f value for spot 5.

Give your answer to **two** significant figures.

$$\text{spot 5} = 52 \text{ mm}, \text{ solvent front} = 119 \text{ mm}$$

$$R_f = 52 \div 119 = 0.437$$

$$R_f = \dots\dots\dots 0.44 \dots\dots\dots [2]$$

- (iii) The student had a table of R_f values. However, these values were determined using a different solvent mixture than the one used by the student.

Explain why using this table of values might lead to incorrect identification of the pigments.

The distance moved by each pigment depends on its solubility in the solvent mixture used.
So the pigments would have different R_f values in a different solvent mixture, and the values would not match.

[2]

(c) When light energy is absorbed by photosynthetic pigments it causes electrons to be excited to a higher energy level.

(i) Explain how energy from the excited electrons is transferred to ATP.

Excited electrons pass along the electron transport chain (ETC) in the thylakoid membrane through a series of redox reactions, losing energy. Each electron carrier is at a lower energy level than the one before. The energy released is used to pump protons (H^+) from the stroma into the thylakoid space, creating a proton (electrochemical) gradient. Protons diffuse back through ATP synthase (chemiosmosis), and the kinetic energy of the moving protons is converted to the chemical energy of ATP ($ADP + P_i$).

[4]

(ii) Suggest **two** reasons why the transfer of energy from excited electrons to ATP is not 100% efficient.

1. Some energy of the electrons is lost as heat.
2. Leakage of protons across the thylakoid membrane, not through ATP synthase.

[2]

4 (a) Fig. 4, on the Insert, shows a photomicrograph of a blood smear.

(i) Identify the three different types of leucocytes labelled **A**, **B** and **C** on Fig. 4.

A ..neutrophil.....

B ..monocyte.....

C ..lymphocyte.....

[3]

(ii) State one structural feature of the cell labelled **D** in Fig. 4 that is different to other eukaryotic cells.

..It has no nucleus (is anuclear); it is a biconcave disc..... [1]

(b) A sample of blood was diluted by taking 0.001 cm^3 of blood and making up to a total volume of 1.5 cm^3 .

The number of erythrocytes in the diluted sample was counted using a haemocytometer of depth 0.1 mm .

There were 15 cells in a 0.2 mm square.

(i) Suggest **one** thing you would do when diluting the blood sample to ensure that the number of erythrocytes is calculated accurately.

..Ensure thorough mixing of the blood and diluent, using an isotonic.....

..diluent..... [1]

(ii) Calculate the total concentration of erythrocytes in the undiluted blood sample.

Give your answer as cells cm^{-3} in standard form.

$$\text{volume} = 0.2 \times 0.2 \times 0.1 = 0.004\text{ mm}^3$$

$$\text{cell density} = 15 \div 0.004 = 3750\text{ mm}^{-3} = 3.75 \times 10^6\text{ cm}^{-3}$$

$$\text{dilution factor} = 1.5 \div 0.001 = 1500$$

$$1500 \times 3.75 \times 10^6 = 5.625 \times 10^9\text{ cells cm}^{-3}$$

concentration = ...5.63..x.10⁹..... cells cm^{-3} [4]

- 5 (a) Fig. 5.1, **on the Insert**, shows an image of a culture of bacteria growing on agar in a petri dish.

- (i) A student concluded that there were two different species of bacteria in the culture.

Evaluate the student's conclusion.

There are clearly different colony morphologies: some colonies are large and orange with rough, irregular edges, others are smooth, round and light brown (yellow), there is a pale cream colony with a wavy edge and many tiny white colonies.

I agree there is more than one type of colony, so more than one species is present.

However, there are more than two types of colony (morphologies), so I disagree: there are probably 3 or 4 (more than two) species present. [3]

- (ii)* Outline a practical procedure to obtain pure cultures of the bacterial species growing on the plate in Fig. 5.1.

In your answer you should include the steps you would take to avoid contamination and confirm the purity of the cultures produced.

Disinfect the work surface and work next to a lit Bunsen burner, using
sterile agar plates (or nutrient broth) and sterile containers.
Select each different type of colony based on its morphology (colour,
size, shape, edge), avoiding colonies that overlap or touch others.
Flame a wire inoculating loop until red hot and let it cool, open the
dish lid only slightly, touch one colony and streak it onto a fresh
sterile agar plate (streak plate) to give isolated colonies. Plate each
colony type on a different plate.
Flame the loop between streaks and flame the necks of any bottles;
do not put lids down on the bench, limit exposure of samples, and
tape the lid on.
Incubate the plates upside down at a suitable temperature, e.g. 25°C,
for 48 hours.
Confirm purity: check that every colony on a plate has the same
morphology, then examine a sample under the microscope with Gram [6]

Additional answer space if required.

staining to check all cells have the same shape and Gram stain colour.
Subculture a single colony again if needed.

(b) Fig. 5.2, **on the Insert**, shows a photomicrograph of bacteria that have been stained with Gram stain.

(i) Explain what can be concluded about the types of bacteria present in this culture.

Both Gram-negative and Gram-positive bacteria are present (at least two types).
Because some cells are stained pink (Gram-negative) and some are stained purple (violet) (Gram-positive).

[2]

(ii) Describe how Gram stain changes the colour of bacterial cells.

Gram-positive bacteria have a thick peptidoglycan wall that binds the crystal violet stain, which is not washed away by alcohol, so they stay purple.
In Gram-negative bacteria the stain is washed away, allowing the safranin counterstain to bind, so they are pink.

[2]

(c) A student investigated the effect of different antibiotics on the growth of bacteria.

They prepared two agar plates:

- Plate 1 was inoculated with a non-pathogenic strain of *E. coli* (a Gram-negative bacterium).
- Plate 2 was inoculated with a non-pathogenic strain of *S. aureus* (a Gram-positive bacterium).

Discs of filter paper were soaked in either an antibiotic solution or distilled water. The discs were then placed on the surface of each plate.

The plates were incubated for 48 hours and the zone of inhibition around each disc was measured.

The results are shown in the table.

Solution on filter paper	Zone of inhibition (mm)	
	Plate 1 (<i>E. coli</i>)	Plate 2 (<i>S. aureus</i>)
Water	0	0
Penicillin	2	16
Polymyxin B	15	1
Tetracycline	15	14

The student made the following conclusions:

1. Penicillin and Polymyxin B are both narrow-spectrum antibiotics.
2. Tetracycline is a broad-spectrum antibiotic.
3. Penicillin is active only against Gram-positive bacteria.
4. Polymyxin B is active only against Gram-negative bacteria.
5. Tetracycline is a bacteriostatic antibiotic whereas penicillin and polymyxin B are both bactericidal antibiotics.

(i) Evaluate the student's conclusions.

Conclusions 1 and 2 are correct, as tetracycline gave large zones on both plates.

Conclusions 3 and 4 may be incorrect: penicillin gave only a 2 mm zone with *E. coli* and polymyxin B only a 1 mm zone with *S. aureus*.

Definitive conclusions cannot be made with only one strain of each type. Conclusion 5 cannot be made from this evidence.

[3]

- (ii) Describe **two** improvements that could be made to the student's method to increase the accuracy of their results.

1. Repeat with more plates for each antibiotic and calculate a mean zone of inhibition.

2. Use the same (controlled) concentration and volume of antibiotic solution, and the same diameter filter paper discs.

[2]

- (iii) Suggest the mechanism of action of penicillin.

Penicillin disrupts the assembly of (destroys) the bacterial peptidoglycan cell wall in Gram-positive bacteria.

[1]

- (iv) Suggest how the mechanism of action of polymyxin B differs from that of penicillin.

Polymyxin B damages the plasma (cell) membrane in Gram-negative bacteria, rather than the cell wall.

[1]

- (v) Outline an experiment to distinguish between a bactericidal antibiotic and a bacteriostatic antibiotic.

Prepare a bacterial lawn on an agar plate, add a disc of antibiotic, incubate, then take a sample from the zone of inhibition and culture it on fresh agar (no antibiotic) to look for evidence that the bacteria have been killed.

Only bactericidal antibiotics show killing (no growth); with a bacteriostatic antibiotic the bacteria grow again.

Repeat with different bacterial species, e.g. Gram-positive and Gram-negative.

[3]

- 6 (a) Fig. 6 shows the reactions of one stage of respiration.

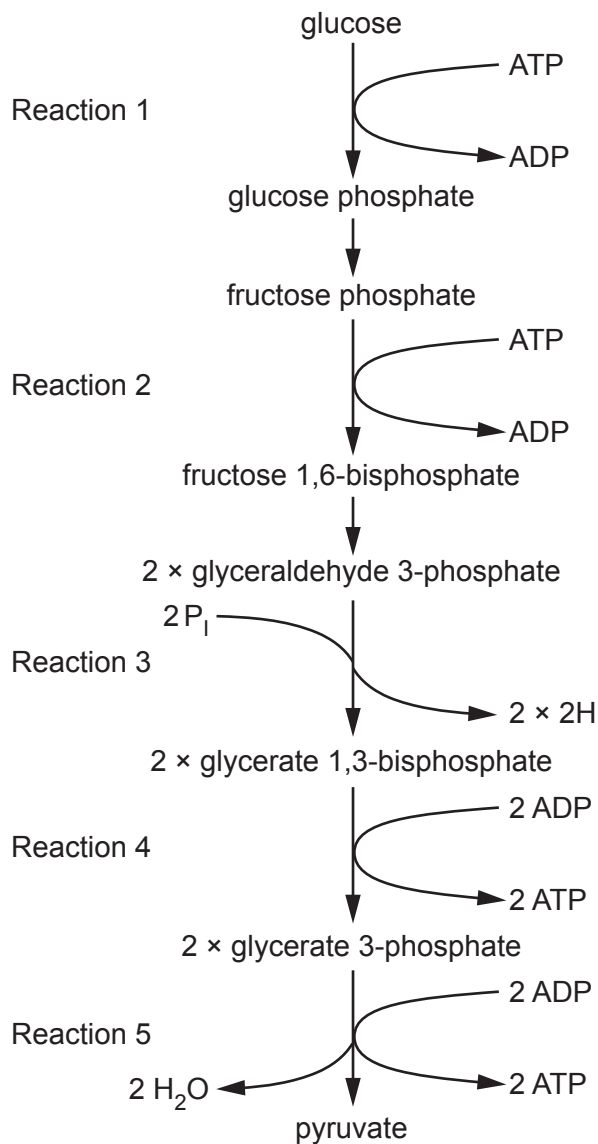


Fig. 6

- (i) Name the stage of respiration shown in Fig. 6.

glycolysis [1]

- (ii) State the location in the cell where the reactions in Fig. 6 occur.

cytoplasm [1]

- (iii) Complete the following table by placing a tick (✓) in the appropriate boxes to show which reactions include substrate level phosphorylation, hydrolysis or a dehydrogenase enzyme.

Reaction	Substrate level phosphorylation occurs	Hydrolysis reaction occurs	Dehydrogenase enzyme is used
1		✓	
2		✓	
3			✓
4	✓		
5	✓		

[3]

- (iv) Reactions 1 and 2 in Fig. 6 illustrate how ATP is used to phosphorylate molecules.

Phosphorylation of glucose prevents it from being able to diffuse out of the cell.

Suggest **one** other effect of glucose phosphorylation that is important in the process shown in Fig. 6.

It increases the energy of glucose, so it reduces the activation energy of the reaction. [1]

- (b) Complete the following passage using the correct words or terms.

In the link reaction, coenzyme A accepts two carbons from *pyruvate* to form acetyl coenzyme A. At the same time *carbon dioxide* and *reduced NAD* are formed. This is an example of oxidative decarboxylation. Acetyl coenzyme A enters the Krebs cycle and reacts with oxaloacetate to form *citrate* which is then converted back to oxaloacetate.

[4]

END OF QUESTION PAPER

This image shows a blank sheet of white paper designed for writing. It features a series of evenly spaced horizontal blue lines across its entire width. A single vertical red line runs down the left side, creating a narrow margin. The paper is otherwise completely empty, with no text or markings.

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