

INTERNATIONAL
BIOLOGY
OLYMPIAD e. V.

IBO



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Student name:	Student code:	Country:
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28th International Biology Olympiad

July 23-30, 2017
University of Warwick
United Kingdom



International Biology Olympiad

Practical Exam 1

BOTANY

**The exam will start and
end with a whistle.**

You have until the exam begins to familiarise yourself with the microscope. You may use one of the slides on your bench.

Total points: 54
Duration: 120 minutes

GENERAL INSTRUCTIONS

In this practical test you have **TWO hours** to do **FOUR Questions**.

You can perform the tasks in any order but note that task 6a** includes a 1 hour incubation step:

Question 1: Plant taxonomy

Task 1 - Identify plant species samples and the ploidy of their tissue. (4 Marks)

Task 2 - Identify flower families and dissect flowers to identify carpel structure. (7.5 Marks)

Task 3 - Prepare stem sections and identify vascular structure and stem organisation. (9 Marks)

Task 4 - Determine the phylogeny of species used in tasks 1-3. (3 Marks)

Question 2: Floral morphology

Task 5 - Dissect flowers to determine floral part identity and flower organisation. (10 Marks)

Question 3: Seed and embryo development

Task 6 - Dissect siliques to determine the stage of seed development and compare seed development in two plants. (10 Marks). **Note this has a **1 hour** incubation step

Question 4: Root morphology

Task 7 - Observe and determine types of root hair and root development in seedlings. (8 Marks)

Task 8 - Determine the manner of inheritance of root development genes. (2.5 Marks)

This is a test of sample handling and manipulation, fine dissection skills, observation and reasoning.

Good luck!

Important Information:

- Please remember to write your name, your student code and your country in the given boxes.
- Write your answers in this question booklet. Only the answers given on this question booklet will be evaluated.
- Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise your flag immediately.
- During experiments, ensure that you handle equipment and samples properly. Any spilled solutions, samples or equipment damaged by you will not be replenished.
- Stop answering and put down your pen immediately when the whistle sounds at the end of the exam.
- Leave the question booklet on your desk at the end of the exam.
- No paper, materials or equipment should be taken out of the laboratory.

MATERIAL AND EQUIPMENT

Make sure that you have received all the materials and equipment listed for each task. If any of these items are missing, please raise your flag.

Required for all questions:

- Stereo (Dissection) Microscope
- Compound microscope
- Hand Lens
- Straight teasing needles
- Curved fine point forceps
- Fine point forceps
- Small scissors
- Waste beakers
- Permanent marker pens
- Roll of white tissue
- Powder free disposable nitrile gloves
- Lab Coat
- Safety Goggles
- 2 blank paper sheets for notes
- Flag to call the lab assistant
- **An English translation of this paper is available upon request.**

Question 1: Plant taxonomy

Plant material

- 7 plant specimens labelled A, B, C, D, E, I, and J
- 5 flowers labelled E, F, G, H, and I (specimens from the same species (E and I) are used in tasks 2 AND 3)
- 3 microscope slides of plant sections labelled K, L, and M

Solutions and reagents

- 10ml Solution A (Toluidine blue stain)
- 10ml Solution B (Distilled water)

Technical material

- Plastic teat pipettes
- Safety razor blades
- 12 microscope slides and 12 cover slips (total for all tasks)
- Disposable jars for used slides/razor blades
- 2 petri dishes
- Timer

Question 2: Floral morphology

Plant material

- 3 tubes containing inflorescence (flowering stem) tips with flowers from 3 different *Arabidopsis thaliana* genetic lines. Labelled O, P, and Q

Question 3: Seed and embryo development

Plant material

- 2 tubes containing inflorescences (flowering stems) from 2 different *Arabidopsis thaliana* genetic lines that are developing seeds. Labelled R, and S.

Solutions

- 1.5 ml Solution C (Hoyer's solution (please note this is an irritant chemical solution))

Technical material

- Electrical tape (yellow)
- Hypodermic needles for dissection
- Safety razor blades
- 12 microscope slides and 12 cover slips (total for all tasks)
- Disposable jars for used slides/razor blades
- Double sided sticky tape (white)
- Timer
- 15 cm ruler

Question 4: Root morphology

Plant material

- 5 agar plates of seedlings: 5 different *Arabidopsis thaliana* genetic lines labelled T, U, V, W, and X

Technical material

- 12 microscope slides and 12 cover slips (total for all tasks)

QUESTION 1: PLANT TAXONOMY

(23.5 marks)

Introduction

As plants have evolved they have acquired morphological characteristics related to the function that they provide. Species can be identified by their characteristics.

Task 1

Species ploidy identification

You are provided with 4 plant specimens labelled **A, B, C & D**. Examine them carefully using the dissecting microscope. Based on your observations complete the Specimen table below using ONE of the options below for each sample. You can use each code more than once in the table.

Specimen table key

Species type	Species code
Angiosperm	1
Moss	2
Conifer	3
Fern	4

Ploidy level	Ploidy code
Haploid: if the tissues or cells in the specimen are from all or part of one type of gametophyte only.	n
Diploid: if the tissues or cells in the specimen are from all or part of one sporophyte generation only.	2n
Tissues or cells from all or part of two sporophyte generations.	2n+2n
Tissues or cells from all or part of one sporophyte generation and two types of gametophytes.	2n+n+n
Tissues or cells in the specimen are from all or part of one sporophyte generation and one type of gametophyte.	2n+n

Specimen table (4 marks)

Specimen	Species code	Ploidy level code
A		
B		
C		
D		

Task 2

Floral structure

You are provided with 5 flowering specimens labelled **E, F, G, H, & I**. Examine them carefully using dissection and the dissecting microscope (only use up to 20x magnification). Based on your observations complete the flower specimen table below using ONE of the options within each column for each sample. You can use each code more than once in the table.

Flower specimen key

Family	Family code
Asteraceae	1
Brassicaceae	2
Lamiaceae	3
Fabaceae	4
Onagraceae (not in syllabus)	5
Magnoliaceae	6

Gynoecium position	Gynoecium code
Hypogynous flower (superior ovary)	7
Epigynous flower (inferior ovary)	8
Perigynous flower (neither superior or inferior ovary)	9

Carpel structure	Carpel code
Single ovary of one carpel only	10
Ovary of fused carpels	11
Many separate carpels (compound flower)	12

Flower specimen table (7.5 marks)

Specimen	Family code	Gynoecium position code	Carpel structure code
E			
F			
G			
H			
I			

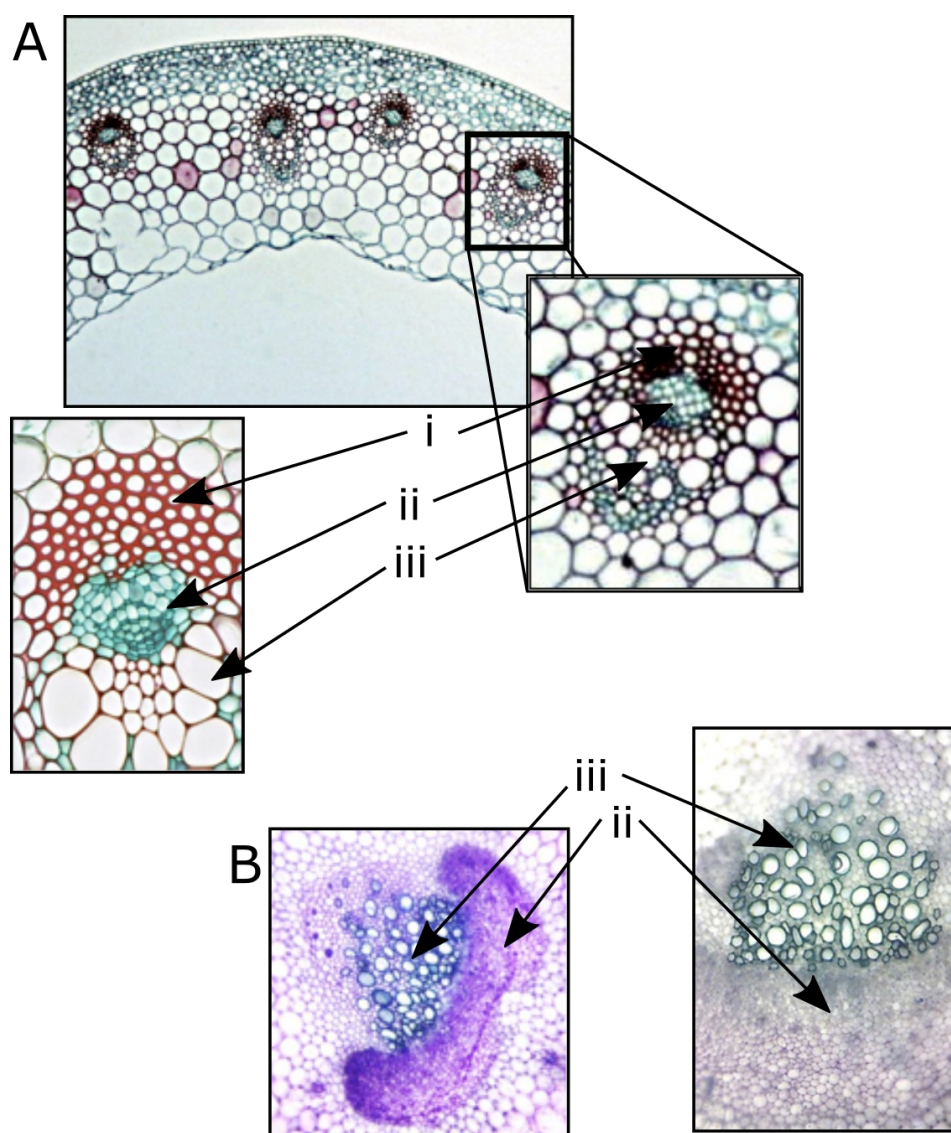
Task 3

Introduction

Higher plants stems are comprised of plant vascular tissues made up of xylem vessels, phloem sieve tube elements and companion cells, and sclerenchyma fibres. Xylem vessels, phloem cells and sclerenchyma fibres can be identified by their structure.

Feature	Xylem vessels	Phloem	Sclerenchyma fibres
Function	Transport of water and mineral ions Structural support	Transport of dissolved carbohydrates	Structural support
Structure	Hollow tubes made of many connected cells	Hollow tubes made of many connected cells	Separate cells joined together to form fibres
Dead/alive	Dead cells	Sieve tube elements kept alive by connections to companion cells	Dead cells
Cell wall	Thickened and possess lignin	No special modifications (plasmodesmata between sieve tube element and companion cells, also sieve plates at the end of cells)	Thickened and possess lignin
Cell contents	Completely hollow – some perforated or slitted end plates of cells remain	Hollow centre with thin layer of cytoplasm around the edge. Sieve plates at the end of each cell.	Hollow dead cells

You can see examples of these cells and the way the vascular tissue is organised in these pictures of transverse sections of buttercup (A) and celery (B).



Transverse sections of stems. i = sclerenchyma fibres, ii = phloem, iii = xylem. A = buttercup, B = celery.

Vascular tissues of the stem are organized in continuous strands in several ways, depending on the spatial location of the cells:

- In **bicollateral** vascular bundle organization the phloem is located towards both the inside and the outside and the xylem is located between the phloem poles.
- In **collateral** vascular bundle organization the phloem is located towards the outside and the xylem is located towards the inside.
- In **concentric amphivasal** vascular bundle organization the xylem encloses the phloem.
- In **concentric amphicribal** vascular bundle organization the phloem surrounds the xylem.
- In **monocotyledons (monocots)**, there are **scattered** vascular bundles, dispersed over the stem, although more towards the periphery
- In **dicotyledons (dicots)**, there are is a **ring** of vascular bundles, arranged in the stem to form a cylinder
- In **woody** species, thickening of the stem occurs, visible as **annual ring** formation.

Stem rigidity is maintained either by turgor pressure inside cells, or secondary wall material (termed secondary thickening).

You are provided with 3 pre-prepared stained transverse stem sections labelled K, L, & M. You are also provided with 3 plant specimens labelled E, I & J (Specimens from the same species (E and I) are used in tasks 2 AND 3). Prepare and stain transverse sections of stems from these plants.

1. Hold the stem of the plant in a vertical angle between the thumb and the index of your non-dominant hand.
2. Hold the razor with your dominant hand and start cutting transverse sections of the stem, as thin as possible.
3. In one petri dish, add a few drops of solution A (blue stain).
4. Place the transverse sections into the stain and stain for 3-5 minutes; staining time depends on the thickness of the section.
5. In a second petri dish, add Solution B (distilled water). Once the staining is completed, transfer the sections into the Solution B, swirl and remove the Solution B. Repeat until excess stain washes out.
6. Carefully transfer the cross-section to a microscope slide, mount in water, add a coverslip over the section and label the slide with the relevant letter (E, I & J).

Examine the transverse sections on both the pre-prepared slides (K,L,M), and those that you have prepared, under low and medium magnification, using both the dissecting and compound microscopes. Based on your observations complete the following table using the ONE code that you think best fits the sample character. You can use each code more than once within the table.

Section specimen key

Vascular bundle type	Type code
Bicollateral	1
Collateral	2
Concentric amphivasal	3
Concentric amphicribal	4

Bundle organisation	Bundle code
Arranged in a ring	5
Scattered	6
Annual rings	7
Stem rigidity	Rigidity code
Turgor pressure	8
Secondary thickening	9

Section table (9 marks)

Specimen	Vascular bundle type code	Vascular bundle organisation code	Provision of stem rigidity code
E			
I			
J			
K			
L			
M			

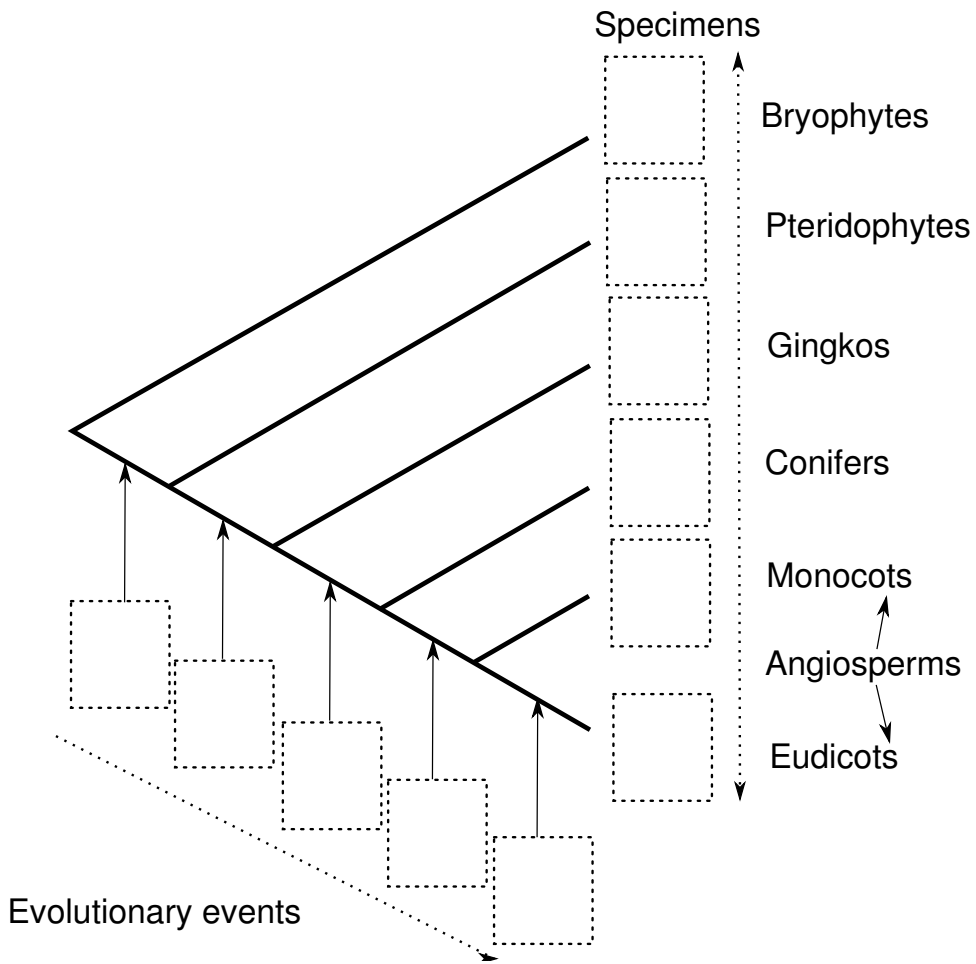
Task 4

Taxonomy

For plant species A-E & I-L, annotate the tree with the most likely position of each specimen species A-E and I-L in the boxes. Each box may include one or more than one specimen, or no specimens.

In addition, annotate the tree with the presence of three evolutionary events (each box may include one or more than one event, or no events):

- I. Development of first flowers
- II. Development of first vasculature
- III. Development of first seeds



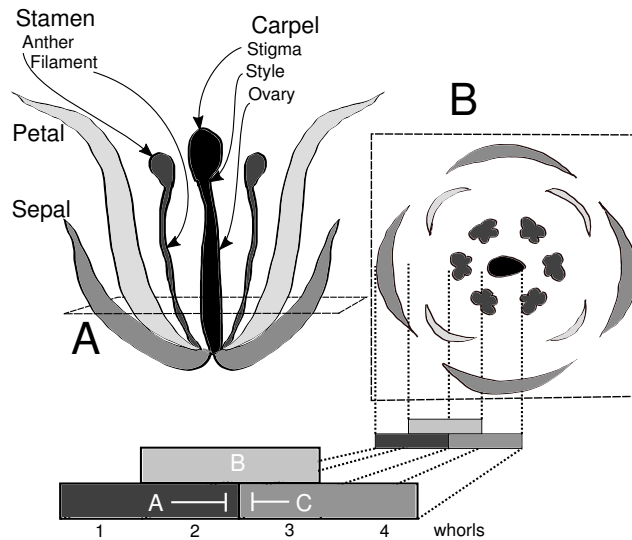
Taxonomic tree (3 points)

QUESTION 2: FLORAL MORPHOLOGY

(10 marks)

Introduction

Wild-type eudicotyledon flowers are comprised of four whorls of floral organs in the following order (outside to inside): sepals, petals, stamens, carpels. The figure below shows you the wild-type floral organ structure of *Arabidopsis thaliana* in longitudinal (A) and cross section (B). The floral organ identity of each whorl is determined by the action or interaction of three classes of 'homeotic' gene activity, A, B, C; as shown.



- Expression of gene A specifies sepals.
- Expression of genes A and B together specifies petals.
- Expression of genes B and C together specifies stamens.
- Expression of gene C specifies carpels.
- Genes A and C repress expression of each other, thus are mutually exclusive.

Task 5a

You have been provided with inflorescence (flowering stem) tips with flowers from three specimens (labelled O, P & Q) from either a wild-type or a knockout mutant *Arabidopsis thaliana* plant. Dissect the flowers and observe the floral organ structures using a range of magnifications. Complete the table below using the codes corresponding to the descriptions below. Choose ONE code for each specimen feature that best describes the phenotype; you can use each code more than once.

Organ identity codes

Organs present	Organs code
Sepals, petals, stamens and carpels	1
Sepals only	2
Stamens and carpels only	3
Sepals and stamens only	4
Sepals and petals only	5
Sepals and carpels only	6

Classes of gene activity	Gene code
A, B, C	7
A, B	8
A, C	9
B, C	10
A	11
B	12
C	13

Genotype	Genotype code
Wild-type	14
Mutant	15

Floral organ identity table (9 marks)

Specimen	Organ identity code	Homeotic gene activity code	Genotype code
O			
P			
Q			

Task 5b

Complete the table below choosing ONE appropriate code for each answer. You can use each code more than once.

Phenotype codes

Phenotype	Phenotype code
Leaves in all four whorls	1
Sepals in all four whorls	2
Petals in all four whorls	3
Carpels in all four whorls	4
Stamens in all four whorls	5

(1 mark)

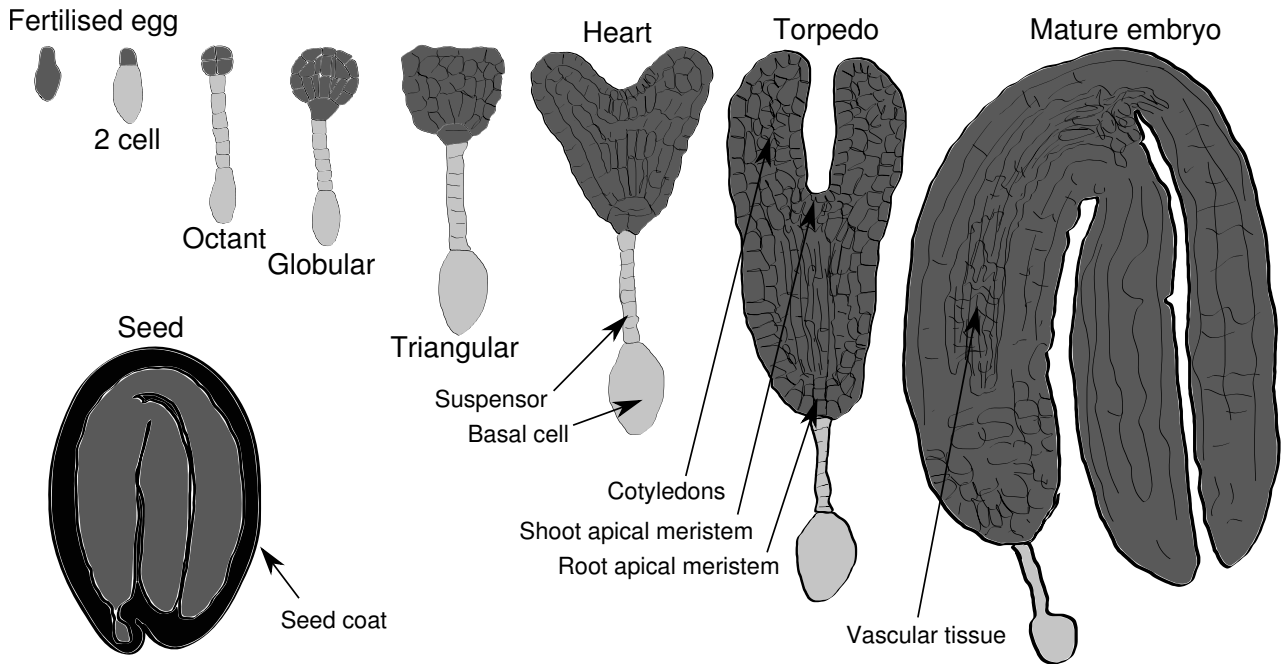
	Phenotype code
What is the flower phenotype in a homozygous knockout BC double mutant?	
What is the flower phenotype in a homozygous knockout ABC triple mutant?	

QUESTION 3: SEED AND EMBRYO DEVELOPMENT

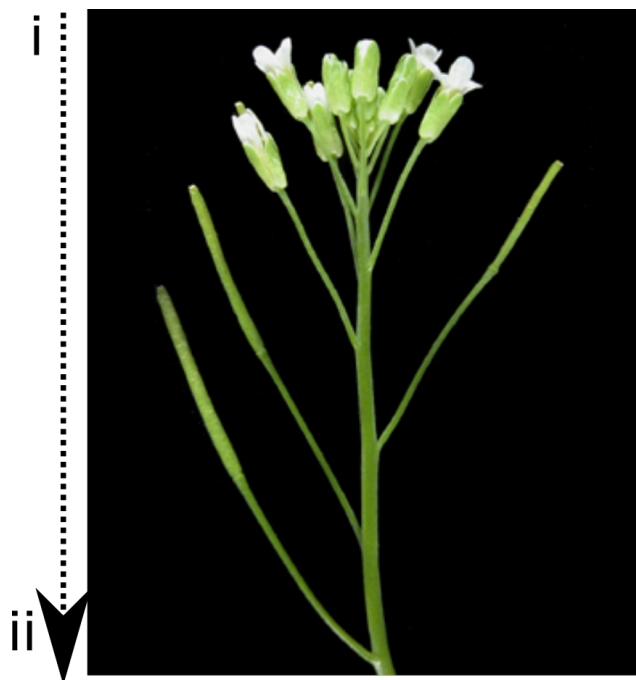
(10 marks)

Introduction

In wild-type plants, after double fertilisation, each ovule in the carpel of *Arabidopsis thaliana* develops into a seed. As the many seeds develop, the carpel structure enlarges and is termed a silique (seed pod). *Arabidopsis thaliana* plant inflorescence (flowering stem) development is indeterminate, thus on a single plant there are many flowers and siliques at a range of developmental stages (see image of *Arabidopsis thaliana* inflorescence below). Within each developing seed, the embryo progressively grows and develops, and a number of stages have been named based on the morphology (see figure below).



Arabidopsis embryo development



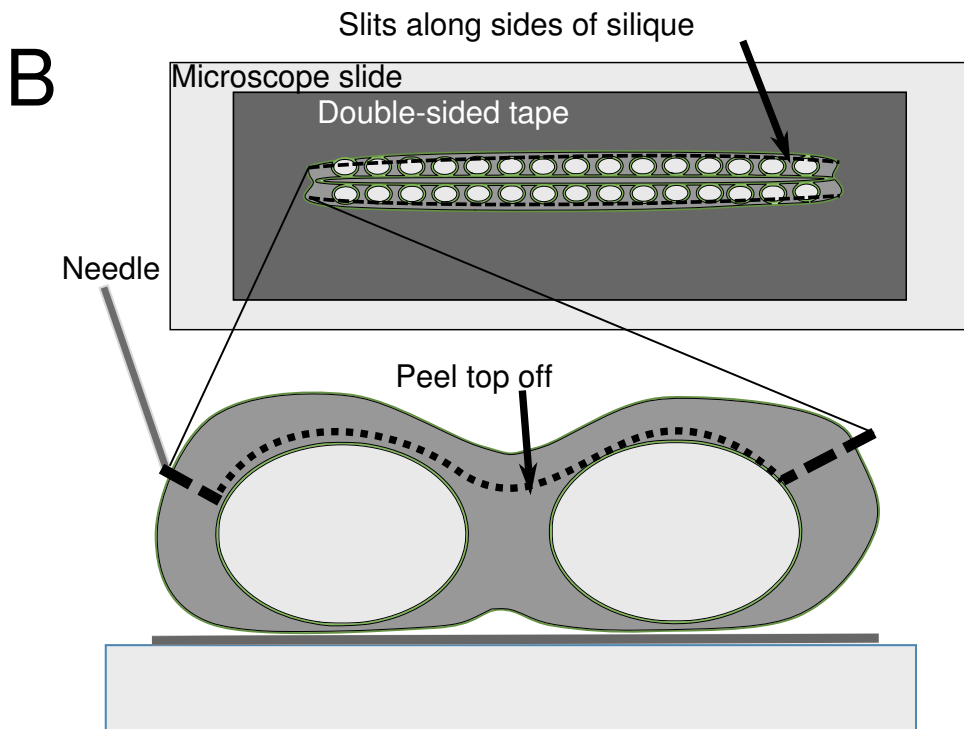
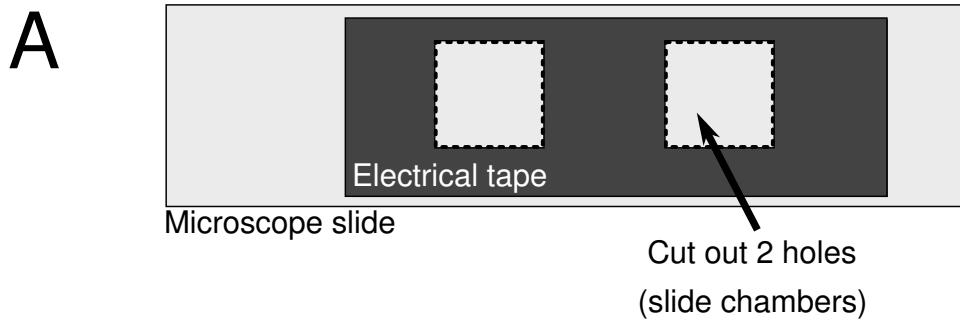
Silique age progression. i = youngest flowers, ii = older embryos and seeds.

Task 6a

You have been provided with 2 tubes containing inflorescences that are developing siliques of *Arabidopsis thaliana* plants labelled R and S. These are different plant lines. Dissect TWO 1.3-1.7cm long siliques from plant R and TWO 1.3-1.7cm siliques from plant S as described below; the silique is attached by a stalk to the plant stem - only measure the silique length. *You must dissect 1.3-1.7cm long siliques as embryo development at this stage will enable you to observe clear differences between plants R and S.*

Silique dissection procedure

1. Make two slide chambers. You will use these in step 4. To do this, first cut a ~4cm strip of the yellow electrical tape and stick it onto a microscope slide. With a razor blade, cut out and remove two small squares from this strip and ensure that the cut edges are flat.
2. Stick a 5cm long piece of double sided sticky tape on to a second microscope slide. Mount the siliques on the sticky tape so that each silique is flat. Observing the slide under a dissecting microscope, use a hypodermic needle to remove the top layer of silique tissue, without damaging the seeds within. Removing this in two strips, on either side of the central silique vein is recommended; the figure below illustrates a cross section of a mounted silique, showing the position of the hypodermic needle for dissection.
3. Observing the slide under a dissecting microscope, record the number of seeds and the number of aborted seeds (if any) within the silique.
4. Wearing gloves, pipette a drop of Solution C (Hoyer's solution) into each slide chamber. *NB Solution C is an irritant so do not get it on your skin.* Using the hypodermic needle or a teasing needle, transfer all of the seeds from each silique into Solution C in the prepared slide chamber; do this under the dissecting microscope, or by eye. Transfer seeds from plant R into one slide chamber, and seeds from plant S into the other slide chamber.
5. Place a cover slip over the seeds. Store the slides at room temperature for **1 hour** to enable the tissue to be cleared (this reaction makes the tissue more transparent so you can see the developing embryos through the seed coat).
6. Observe the samples at high magnification using a compound microscope (e.g. you can see a heart-shaped embryo in this cleared seed).



Silique dissection

Using an average of both siliques dissected, record the number of developing seeds per silique within each plant line in the table below. Then examine the stage of embryo development for the seeds within the siliques. Record the % of seeds at each stage of embryo development.

Silique morphology (4 marks)

Plant	Average number of seeds	% globular stage	% heart stage	% torpedo stage	% mature stage
R					
S					

Task 6b

One of the two lines has abnormal (non wild-type) seed development. Define the abnormality and its frequency. Based on your observation of the number and stage of abnormal embryos, what is the most likely explanation for the abnormal embryos?

Complete the table below, choosing ONE code for each question.

Development codes

Seed defect	Defect code
Embryo pattern formation is disrupted	1
Seeds lack embryos	2
Seeds have multiple embryos	3
Seeds have enlarged embryos	4

Approximate frequencies of abnormal embryos	Approximate frequency code
0%-33%	5
34%-66%	6
67%-100%	7

Cause of embryo abnormality	Cause code
Double fertilisation	8
Development of a non-embryonic cell	9
Spontaneous	10

(6 marks)

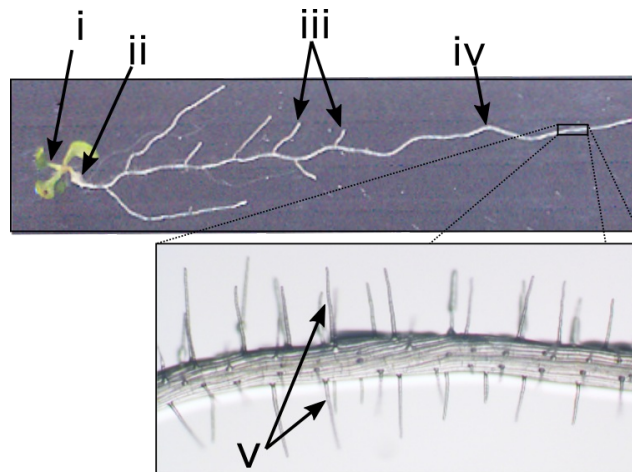
	Which plant line has abnormal development? (R or S)	Defect code	Approximate frequency code	Cause code
Answer				

QUESTION 4: ROOT MORPHOLOGY

(10.5 marks)

Introduction

Plant roots are composed of a primary root and a number of branching or lateral roots. Root hairs are elongations of a subset of root epidermal cells. They develop on both the primary and lateral roots. They have a variety of functions for the plant including providing an increased surface area for nutrient or water uptake, as well as sensing the external environment and gravity.



Root morphology. i = leaves/cotyledons, ii = hypocotyl, iii = lateral roots, iv = primary root, v = root hairs.

You have been provided with 5 agar plates with 5 different *Arabidopsis thaliana* genetic lines.

- Line T is a wild-type line
- Lines U, V, W & X have abnormal root or root hair development/morphology

1. Familiarise yourself with wild-type root and root hair morphology by carefully removing a seedling from one of the plants on plate T and studying it under the dissecting microscope.
2. Mount a section of the root in water on a microscope slide, add a coverslip and observe the density and length of the root hairs under the compound microscope.

Task 7a

Analyse lines U, V, W & X by following the same procedure. Complete the table with ONE code that best describes the phenotype that you observe. You can use each phenotype more than once.

Root phenotype codes

Root phenotype	Code
Short root hairs	1
No root hairs	2
Longer root hairs	3
Fewer root hairs	4
More root hairs	5
Shorter lateral roots	6
Longer lateral roots	7
No lateral roots	8

Root phenotypes (6 marks)

Specimen	Root development phenotype code
U	
V	
W	
X	

Task 7b

Observe the wild type seedling (T) in the root hair zone. Choose ONE statement that best describes the pattern of the epidermal cells that are root hairs. Mark this statement with a cross (X) in the table below.

(2 marks)

Root hair formation pattern	X the correct statement
Root hairs are in rings of rows of epidermal cells.	
Root hairs form from random cells.	
Root hairs are in files or columns of epidermal cells.	
Root hairs form a checkerboard pattern on the epidermis.	

Interpretation of your results

Plants have two copies of each gene (one on each chromosome from each parent). The wild type and mutant plants we have given you contain the same copy of each gene on both chromosomes (homozygous). If a plant contains two different copies (alleles) of a gene it is heterozygous.

Root hairs develop from surface (epidermal) cells but not all epidermal cells develop root hairs. One gene (called *WEREWOLF*) enables a root hair to develop, and other genes determine how long root hairs grow.

Task 8a

If a plant was homozygous for a knockout mutation in the *WEREWOLF* gene (*werewolf*) AND also homozygous for another knockout mutation that usually caused extra-long root hairs, what type of root hairs would you expect to see on the plant? Mark the ONE correct answer with a cross (X) in the table.

(0.5 marks)

Root hair phenotype	X the correct statement
Normal length root hairs	
No root hairs	
Extra long root hairs	
Long root hairs	

Task 8b

Complete the Punnet square for the expected genotypes of a cross between a *WEREWOLF* wild-type (BB) plant and a *werewolf* recessive homozygote (bb).

(0.5 marks)

<i>blank</i>	Wild-type	Allele 1	Allele 2
Recessive homozygote	<i>blank</i>		
Allele 1			
Allele 2			

Two other plants were crossed and the progeny had the phenotypes shown in the table.

Phenotype	Number of progeny
Normal roots	24
No root hairs	8

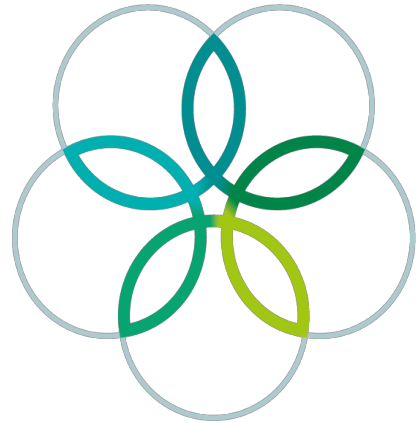
Fill in the table about this cross.

(1.5 marks)

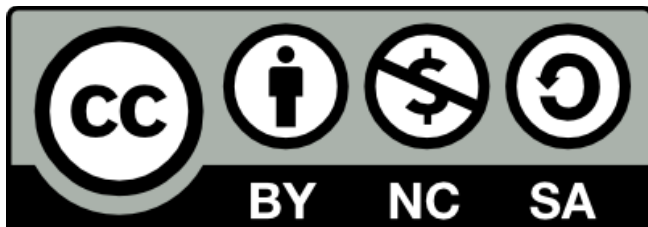
What were the genotypes of the two plants that were crossed (in terms of B or b)?	?? x ??
How many progeny have the genotype Bb?	
How many progeny have the genotype BB?	

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28th International Biology Olympiad

July 23-30, 2017
University of Warwick
United Kingdom



International Biology Olympiad

Practical Exam 2
BIOCHEMISTRY

**The exam will start and
end with a whistle.**

Total points: 78
Duration: 120 minutes

GENERAL INSTRUCTIONS

In this practical test you have **TWO hours** to do **THREE Questions**.

You should perform the tasks in the order given here:

Question 1: Analysis of blood markers (11 marks). This section should take approximately 15 minutes to complete. You will be provided with some data to analyse.

Question 2: Practical determination of kinetic parameters (60 marks). This section should take approximately 90 minutes to complete. You will be generating your own data.

Question 3: Analysis of genetic markers (7 marks). This section should take approximately 5 minutes to complete. You will be provided with some data.

In this exam you will analyse a patient history through blood marker characteristics, enzyme kinetics and family inheritance of a genetic disorder.

Good luck!

Important Information:

- Please remember to write your name, your student code and your country in the given boxes.
- Write your answers in this question booklet. Only the answers given in this question booklet will be evaluated.
- Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise your Green card immediately.
- During experiments, ensure to handle equipment properly. Any spilled solutions or equipment damaged by you will not be replenished.
- Stop answering and put down your pen immediately when the whistle sounds at the end of the exam.
- Leave the question booklet on your desk at the end of the exam.
- No paper, materials or equipment should be taken out of the laboratory.
- **An English translation of this paper is available upon request.**

MATERIALS

- Ice Box
- Solution A: 2 x Concentration Reaction Master Mix containing: 50 mM potassium phosphate buffer, pH 7.6; 14 mM MgSO₄; 2.64 mM ADP; 40 U Lactate Dehydrogenase; 0.4 mM NADH (10 ml) (in ice box)
- Solution B: H₂O (10 ml)
- Solution C: 10 mM phosphoenol pyruvate (PEP) in 0.1 M potassium phosphate buffer, pH 7.6 (1.5 ml) (in ice box)
- Diluted plasma samples F, M and D (0.5 mL each) (in ice box)
- A 20-200 µl pipette
- A 100-1000 µl pipette or 200-1000 µl pipette
- Pipette tips, to suit pipettes
- 1 ml plastic cuvettes (1 cm path length) x 20
- Waste tip and cuvette disposal vessel
- A visible light spectrophotometer - check the Spectrophotometer Guide sheet, included as a separate document, for instructions on using your spectrophotometer
- a cuvette will be placed in sample chamber to indicate the correct orientation.
- A Cuvette stand
- Parafilm® squares x 30
- Digital Timer
- 30 cm ruler
- Scientific Calculator
- Pen
- Pencils

INTRODUCTION

Important Background Information

Pyruvate kinase (PK), a 58 kiloDalton (kDa) protein, functions as a homotetramer and has a pivotal role in the glycolytic pathway (Fig. 1), converting its substrate phosphoenol pyruvate (PEP) into pyruvate in a reaction that also generates ATP.

PK deficiency (PKD) is the commonest cause of hereditary non-spherocytic haemolytic anaemia, a group of genetically inherited diseases associated with a net loss of red blood cells. In the PKD disorder, red blood cells are broken down (undergo haemolysis) prematurely, resulting in a shortage of red blood cells (anaemia). In hereditary non-spherocytic haemolytic anaemia, the red blood cells do not assume a spherical shape as they do in some other forms of haemolytic anaemia. Blood analysis can also be helpful in understanding the PKD disease. In addition, the disease is often associated with reticulocytosis; an increase in number of reticulocytes (immature red blood cells).

PKD is inherited in an autosomal recessive pattern. The parents of an individual with an autosomal recessive condition may, or may not, show signs and symptoms of the condition depending on the alleles that they possess.

Given equal subunit expression and random association, PK in either simple heterozygotes or compound heterozygotes (heterozygotes where there are two different recessive alleles) is a spectrum of heterotetrameric isozymes (A4, A3B, A2B2, B3A, B4) in a theoretic ratio of 1:4:6:4:1. If B is a variant and A is normal, some 94% of all PK in PKD patients contains one or more mutant subunits; in vivo, non-assembled monomers are prone to protease digestion. It is important to note that specific alleles code for PK proteins that form tetramers, whilst other alleles code for PK proteins that remain as monomers.

Clinical symptoms usually observed in simple heterozygotes, compound heterozygotes and homozygotes are variable, ranging from neo-natal jaundice requiring blood transfusions, to haemolytic anaemias that are self-regulated and show minimal clinical signs. Many of the mutant PKs have been identified on the basis of the biochemical characteristics of the defective enzyme.

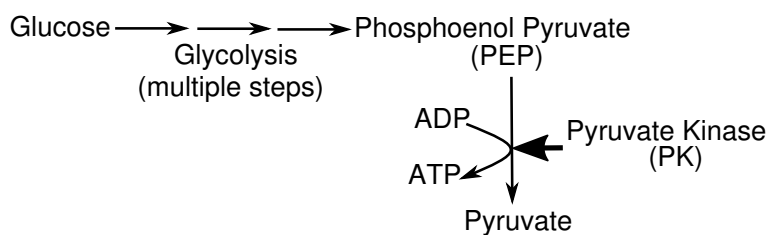


Figure 1: The role of pyruvate kinase (PK)

A clinical scenario:

Various family members have been diagnosed with non-spherocytic haemolytic anaemia, perhaps associated with PK deficiency. Numerous tests can be conducted including blood work to determine the parameters of the blood composition (haemogram) and an enzyme analysis of plasma specifically for PK activity. Additionally, a genetic analysis including identification of PK proteins from individual members and an analysis of a family pedigree to confirm the likely inheritance pattern can be undertaken.

You will analyse and collect data from the 3 family members: Father (F), Mother (M) and Daughter (D), in order to determine the family pedigree.

QUESTION 1

Blood Analysis:

Whole blood samples were collected from the Father (F), Mother (M) and their Daughter (D). After blood is taken and analysed the following partial results were obtained (Table 1):

Table 1

	Patient F (Father)	Patient M (Mother)	Patient D (Daughter)
Red Blood Cells (RBCs) count	$5.35 \times 10^{12}/l$	$3.65 \times 10^{12}/l$	$3.01 \times 10^{12}/l$
White Cell count	$7.5 \times 10^9/l$	$4.2 \times 10^9/l$	$3.4 \times 10^9/l$
Haemoglobin concentration	14.4 g/dl	10.5 g/dl	8.5 g/dl
Reticulocytes (% of RBCs)	1.67 %	2.63 %	10.46 %

Various analyses can be undertaken, including the determination of the haematocrit, the packed red blood cell volume, given as a percentage of total blood volume. The haematocrit is produced by taking up a sample of blood into a micro-capillary tube and centrifuging the sample so that the red blood cells become packed. The percentage of red blood cells is calculated by measuring the packed volume as a function of the total volume in the capillary tube.

Task 1a

Determine the haematocrit (% packed red blood cells by volume) shown in Figure 2, for each individual. Include these data in the first row of Table 3. This is expressed as a percentage to one decimal place (d.p.).

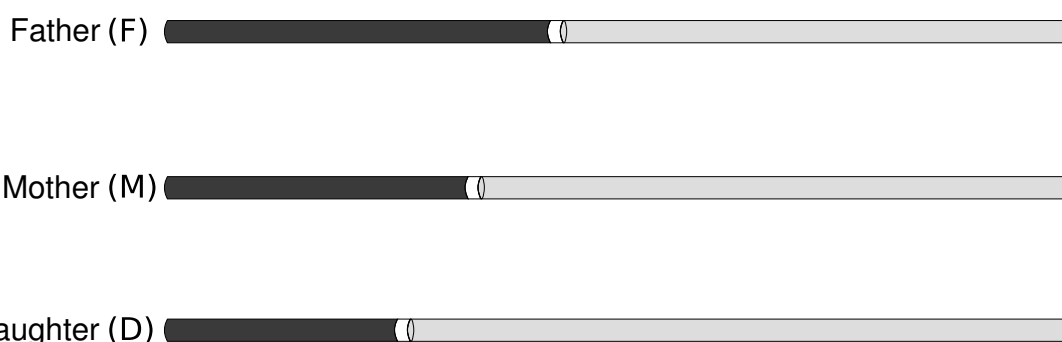


Figure 2 Haematocrit diagrams from the Father, Mother and Daughter. The figure shows diagrammatic representations of the haematocrit, depicting a capillary tube of total sample containing red blood cells (left hand side) white blood cells (buffy coat) (between RBC and plasma) and plasma (right hand side).

Table 2: Normal Values for Haemogram of healthy people

	Male	Female
Haemoglobin (g/dl)	13.5 - 17.5	11.5 - 15.5
Haematocrit (%)	40.0 - 52.0	36.0 - 48.0
Red blood cell Count ($\times 10^{12}/l$)	4.50 - 6.50	3.90 - 5.60
Mean Cell Haemoglobin (MCH) (pg)	26.0 - 34.0	26.0 - 34.0
Mean Cell Volume (MCV) (fl)	78.0 - 95.0	78.0 - 95.0
Mean Cell Haemoglobin Concentration (MCHC) (g/dl)	30.0 - 35.0	30.0 - 35.0
White Blood Cell count ($\times 10^9/l$)	4.0 - 11.0	4.0 - 11.0
Platelet Count ($\times 10^9/l$)	105 - 450	105 - 450
Reticulocyte Count (%)	0.5 - 1.5	0.5 - 1.5
PK activity (U/g Haemoglobin)	11.8 - 18.6	11.8 - 18.6

Task 1b

Using the packed cell volume from Figure 2, and the patient data from Table 1, calculate the following parameters for each patient (F, M and D):

The red blood cell volume, known as the **Mean Cell Volume (MCV)**,

The mass of haemoglobin per red blood cell, known as the **Mean Cell Haemoglobin (MCH)**,

The concentration of haemoglobin per red blood cell, known as the **Mean Cell Haemoglobin Concentration (MCHC)**.

The MCV is given in femtolitres (fl) (fl is 1×10^{-15} l) to one d.p. MCH is expressed in picograms (pg) (pg is 1×10^{-12} g) to one d.p. MCHC is the total concentration of haemoglobin in the RBC fraction. This is expressed as g/dl (deci litre) (1×10^{-1} l) to one d.p

Write the numbers in Table 3 for the Father (F), Mother (M) and Daughter (D).

Show your calculations for each parameter, in Box 1 for the Father (F).

Box 1: Calculations for patient F (Father) (4 marks)

Element	Show your working here
Haematocrit	
MCV	
MCH	
MCHC	

Table 3 (4 marks)

	Sample F (Father)	Sample M (Mother)	Sample D (Daughter)
Haematocrit (%)			
MCV (fl)			
MCH (pg)			
MCHC (g/dl)			

Further analysis

Anaemias are classified, according to the size of the red blood cell, as being either normocytic (normal MCV), macrocytic (increased MCV) or microcytic (decreased MCV). Microcytic anaemias are also often described as being hypochromic based on peripheral whole blood smear examination. The optical properties of the small, thin microcytes make them appear hypochromic on the blood smear, while the haemoglobin concentration remains in the normal range (microcytic; hypochromic anaemia). Normochromic blood smears show no change in optical properties on the blood smear with the haemoglobin concentration remaining in the normal range.

Table 2 gives the normal ranges for MCV, MCH and MCHC.

Task 1d

Using the values you have obtained (included in Table 3.), establish the classification in terms of size of blood cells and levels of haemoglobin of the blood of sample F, M and D.

You should answer from one of the following options for each patient (N.B. you can use any of the options more than once):

- A. Macrocytic, normochromic
- B. Microcytic, hypochromic
- C. Normocytic, normochromic

(3 marks)

Patient	Classification (A, B or C)
Father (F)	
Mother (M)	
Daughter (D)	

QUESTION 2

Assay of pyruvate kinase activity

Biochemistry can be utilised to determine properties of well characterised enzymes by studying the variation in their kinetics. Enzyme activity is usually determined by monitoring the disappearance of a substrate or the appearance of a product, often using changes in spectrophotometric properties of the reaction mixture.

PK is conveniently assayed by a **coupled assay** in which the product of the reaction, pyruvate (Equation 1), is used as substrate for the NADH-linked enzyme lactate dehydrogenase (LD) (Equation 2), which is added to the reaction mixture in sufficient amounts to convert all of the pyruvate produced during the reaction to lactate. The reaction can be monitored by following the decrease in absorbance at 340 nm over time. The equations for the coupled reaction is shown (Fig. 3).

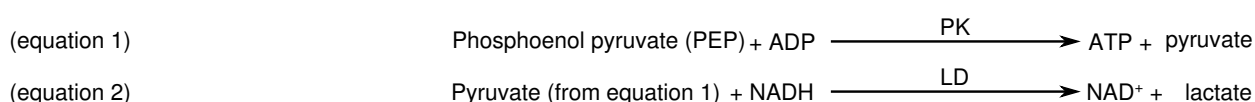


Figure 3. Pyruvate kinase and Lactate dehydrogenase-catalysed breakdown of Phosphoenol pyruvate (PEP) (Equation 1) and Pyruvate (Equation 2)

An enzyme's kinetic properties can be determined by varying the PEP substrate concentration (Equation 1).

Aims of the experiment

To determine the functionality of PK from the three patient plasma samples, Father (F), Mother (M) and the Daughter (D).

- Determination of activity of PK in patient plasma samples through the use of the coupled assay.
- Calculation of the kinetic properties of PK in absolute units.

Methods: pyruvate kinase assay

Before starting the assay, please note the following:

1. Solution A, C and patient samples F (father), M (mother) and D (daughter) should be kept on ice, whilst Solution B (H₂O) should be kept at room temperature.
2. The spectrophotometer should be blanked against water (provided) at 340 nm.
3. Make up the reaction mix by pipetting the reagents shown in Table 4 directly into a plastic 1 ml cuvette. You should add all the components *except* the plasma sample, *then* add the plasma sample last when you are ready to start the reaction.

Table 4. Pyruvate kinase assay components

You are provided with the following reagents	
Solution A	2x stock concentration Reaction Master Mix (10 ml) (the working concentration for the Reaction Master Mix is 1x)
Solution B	H ₂ O (10 ml)
Solution C	10 mM phosphoenol pyruvate (PEP) (1.5 ml)
Diluted patient plasma labelled F (Father), M (mother), or D (Daughter)	Diluted plasma from each patient (0.5 ml each)

Task 2a

Calculate the reaction volumes for FIVE concentrations of PEP (Solution C) (choose FIVE concentrations ranging from 0.2 mM to 1.5 mM). Show an example of your calculations for the highest concentration in Box 2.

PEP will drive the reaction and allow the determination the Pyruvate Kinase activity in each plasma sample (from Father (F), Mother (M) and Daughter (D)).

Box 2 (1 mark)

Show an example of your workings for the calculation of volumes to be used of the highest PEP concentration.

Task 2b

Write the volumes used to make the reaction mixes into the blank spaces in Table 5; note that these will be the same for each plasma sample (F, M and D).

Table 5, Reaction Volumes. You should write the substrate concentrations, PEP [S], you have decided to use in the first row at the top of each column, and the volumes of each solution in the respective boxes. (3 marks)

Concentration	1	2	3	4	5
PEP concentration					
Solution A (µl)					
Solution B (µl)					
Solution C (µl)					
Plasma	50 µl	50 µl	50 µl	50 µl	50 µl
Final volume	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl

The PK activity Assay

Mix contents by inverting of the cuvette (making sure there is a piece of parafilm held in place over the opening using your thumb or forefinger), take the A_{340} reading at time zero, then record the A_{340} at 30 second intervals for 90 seconds. Record your absorbance values to the number of decimal places as displayed on your spectrophotometer.

For each sample you need to determine the initial rate of reaction $\Delta A/\Delta t$ (time expressed in minutes) for the various concentrations of PEP ([S]) you used, using your time zero value and another value of your choice. Highlight the time points which you have used to calculate the rate by circling the values.

Task 2c

Perform the experiment as described and record the absorbance readings using the tables provided (Tables 6.1, 6.2 and 6.3).

Table 6.1 Sample F (6 marks)

Concentration	1	2	3	4	5
[S]					
Absorbance at 0s					
Absorbance at 30s					
Absorbance at 60s					
Absorbance at 90s					
Rate ($\Delta A/\Delta t$) (min^{-1})					

Table 6.2 Sample M (6 marks)

Concentration	1	2	3	4	5
[S]					
Absorbance at 0 s					
Absorbance at 30 s					
Absorbance at 60 s					
Absorbance at 90 s					
Rate ($\Delta A/\Delta t$) (min^{-1})					

Table 6.3 Sample D (6 marks)

Concentration	1	2	3	4	5
[S]					
Absorbance at 0 s					
Absorbance at 30 s					
Absorbance at 60 s					
Absorbance at 90 s					
Rate ($\Delta A/\Delta t$) (min^{-1})					

Calculation of Kinetic Parameters

It is possible to express a change in absorbance over time as a change in product/substrate concentration. For each initial rate, $\Delta A/\Delta t$, calculate the initial velocity (v_0) ($\Delta \text{Conc}/\Delta t$) of the reactions for each PEP substrate concentration ([S]). The absolute units for velocity should be given as $\mu\text{mol min}^{-1}$ using the Beer-Lambert law. The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species.

The equation is: $A = \epsilon lc$

A = Absorbance

ϵ = molar extinction coefficient

l = path length of light

c = concentration

The reaction in this experiment measures the conversion of NADH, which has a molar extinction coefficient of $6220 \text{ L mol}^{-1} \text{ cm}^{-1}$, to NAD^+ (which has a negligible absorbance at 340 nm), and therefore the conversion of PEP to lactate. As there is a 1:1 molar ratio of components the conversion of NADH is therefore equal to the conversion of PEP. The cuvette has a path length of 1 cm.

Task 2d

Calculate the initial velocity (v_0) and write your answers in the tables below (Table 7.1, 7.2 and 7.3) for each patient (Father (F), Mother (M) and Daughter (D)). Your values should be given to the nearest 3 decimal places (d.p.).

Table 7.1 Patient F (1 mark)

[S]	v_0

Table 7.2 Patient M (1 mark)

[S]	v_0

Table 7.3 Patient D (1 mark)

[S]	v_0

Calculation of enzyme parameters K_M and V_{max}

By using the different rates of reaction for differing substrate concentrations two enzymatic parameters can be determined: K_M and V_{max} . These two parameters can be estimated by plotting the data in one of two ways: a Michaelis-Menten plot (Figure 4 A) or a Hanes-Woolf plot (Figure 4 B).

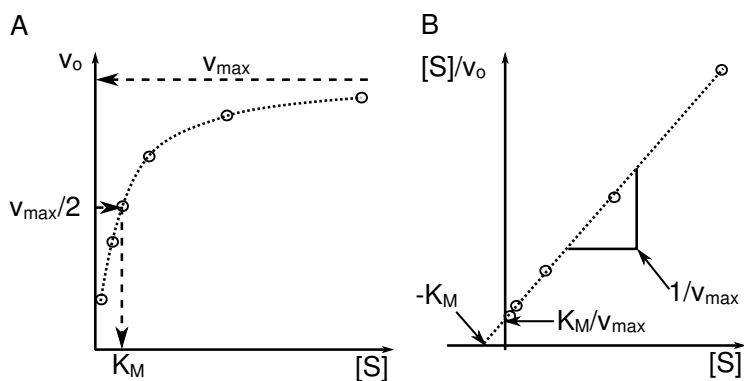
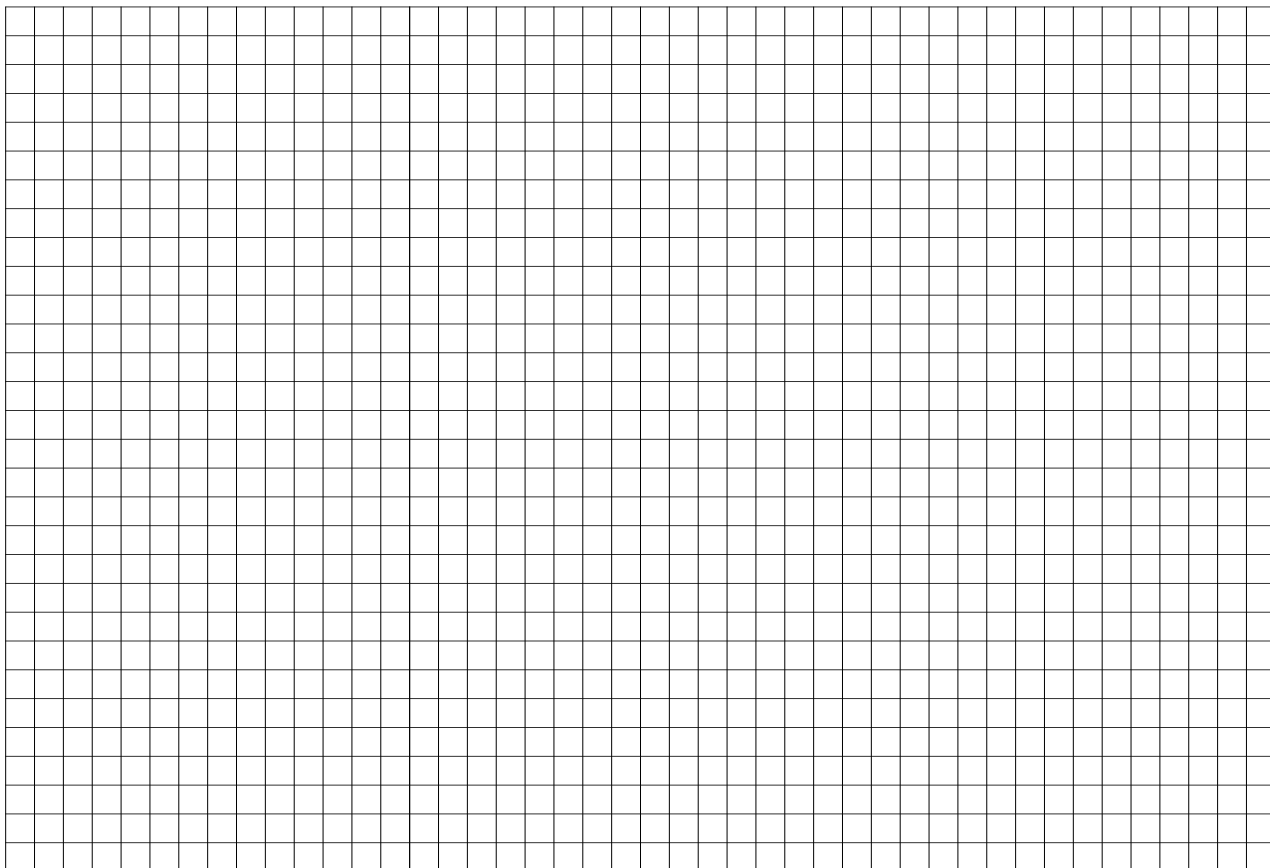


Figure 4. Plotting kinetic data. A. Michaelis-Menten plot of initial reaction rate (v_0) over substrate concentration $[S]$. K_M is the substrate concentration that gives half maximal reaction rate. V_{max} is the maximal rate where $[S]$ is saturating. **B.** Hanes-Woolf plot of the ratio of the substrate concentration and initial rate ($[S]/v_0$) over the substrate concentration. K_M can be determined from the x-axis intercept of the best linear fit to the data points, and V_{max} can be determined from the slope.

Task 2e

Use your data to construct a Michaelis-Menten plot of v_0 over $[S]$ for each patient (F, M and D) on the graph area below. You should draw one graph with three data plots; one each for Father (F), Mother (M) and Daughter (D). Use a cross (X) to mark data from the Father (F), a circle (O) to mark data from the Mother (M) and a triangle (Δ) to mark data from the Daughter (D).



Michaelis-Menten plot (10 marks)

Task 2f

Using the three data plots, estimate V_{\max} and K_M ; write the estimates in the table below using the correct units and to the nearest 2 decimal places (d.p.), for each sample from the graph you have drawn.

(6 marks)

Patient	V_{\max}	K_M
Father (F)		
Mother (M)		
Daughter (D)		

Task 2g (3 marks: one mark for each completed table)

Determine $[S]/v_0$ for each substrate concentration and each sample and write your answers in the tables below for each patient (Father (F), Mother (M) and Daughter (D)). Your values should be given to the nearest 1 decimal places (d.p.).

Patient F (1 mark)

[S]	[S]/v0

Patient M (1 mark)

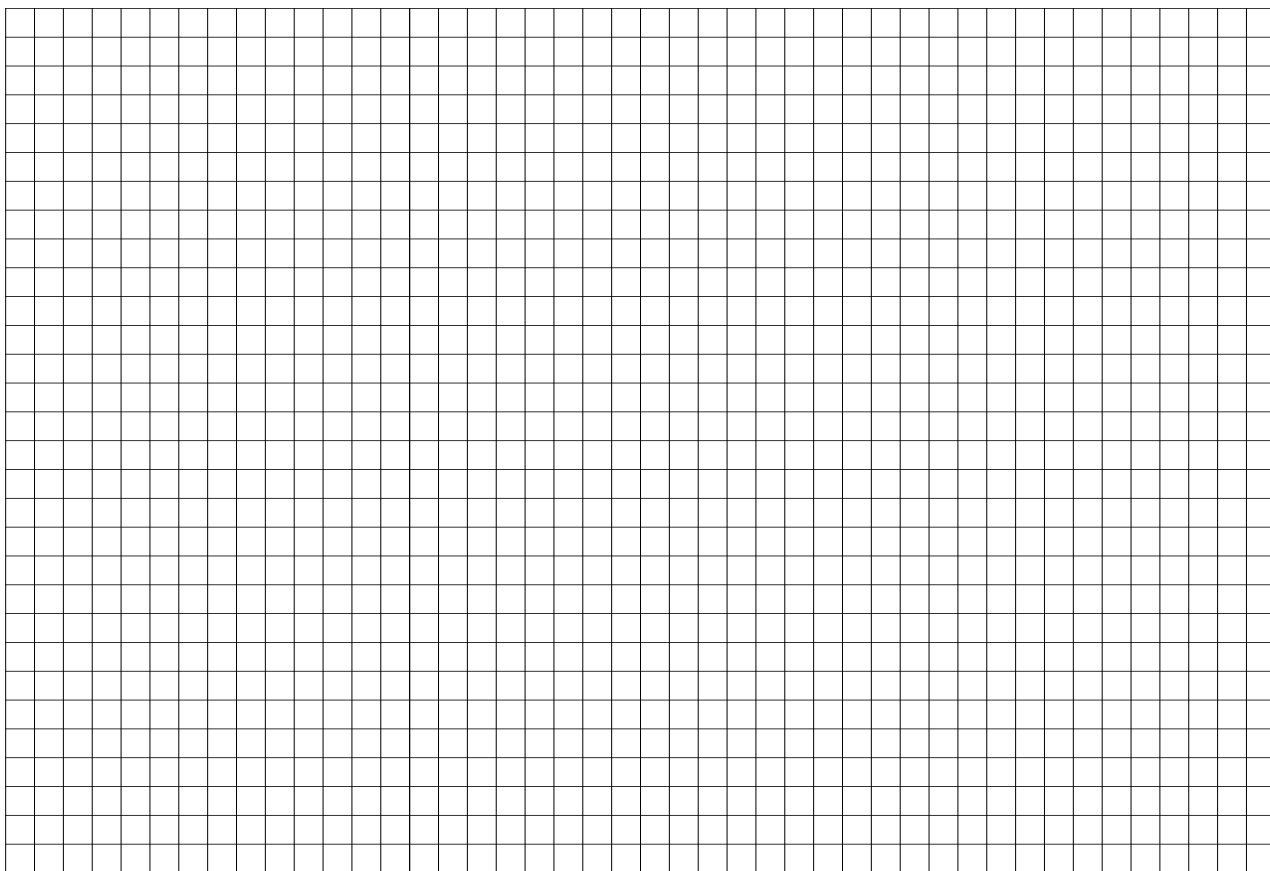
[S]	[S]/v0

Patient D (1 mark)

[S]	[S]/v0

Task 2h

Plot $[S]/v_0$ over $[S]$ for each patient (F, M and D) on the graph area below. You should draw one graph with three data plots; one each for Father (F), Mother (M) and Daughter (D). Add a line of best fit for each data plot. Use a cross (X) to mark data from the Father (F), a circle (O) to mark data from the Mother (M) and a triangle (Δ) to mark data from the Daughter (D).



Hanes-Woolf plot (10 marks)

Task 2i

Calculate V_{\max} and K_M for each patient and include in the table below using the correct units to two decimal places (d.p.).

(6 marks)

Patient	V_{\max}	K_M
Father (F)		
Mother (M)		
Daughter (D)		

QUESTION 3

Genetic Analysis and Diagnosis

Blood samples from the father, mother and daughter were sent to a clinical testing laboratory for further analysis. Proteins were extracted from whole blood, before separation on a polyacrylamide denaturing electrophoresis gel. The samples were visualised, after transferring the proteins to a nitrocellulose membrane (western blot), by detection using enzyme-conjugated anti-PK antibodies and subsequent chemiluminescence. The resulting visualisation of the PK protein content is shown in the image in Figure 5. Please refer to the Important Background Information to help interpret the figure.

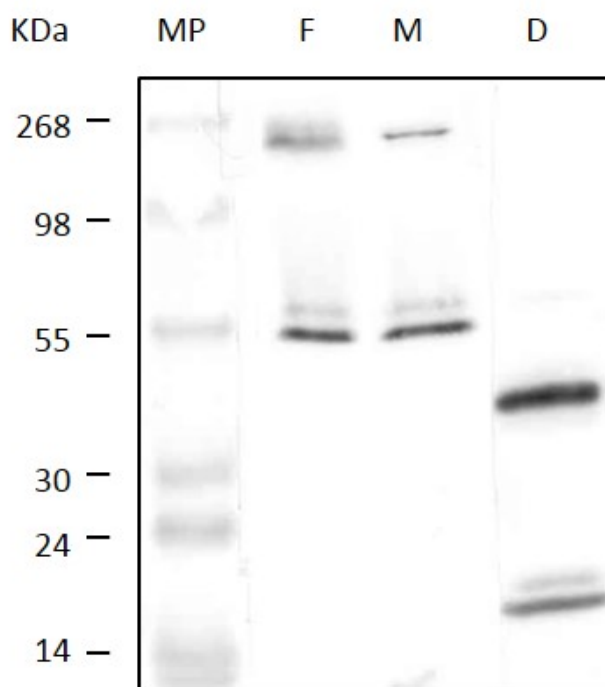


Figure 5. Immunoblot of PK variants in the affected family. Red blood cell lysates from the father (F), the mother (M) and the daughter (D) were separated by Polyacrylamide gel electrophoresis (PAGE), transferred to membrane (western blot), and probed with anti-PK antibodies (immunoblotted). Sizes and migration of marker proteins (MP) are indicated on the left.

Task 3a

Complete the table below, indicating which size protein bands are visible for each patient (F, M or D), as either 'present' or 'not present'. Use the letter P to mark present, and NP to mark not present.

Task 3b

Indicate on the table whether any of the three patients (F, M or D) contain a PK tetramer by marking as either 'present' or 'not present'. Use the letter P to mark present, and NP to mark not present.

(5 marks)

Molecular weight	Patient F	Patient M	Patient D
232 kDa			
58 kDa			
40 kDa			
18 kDa			
PK tetramer			

The clinical testing laboratory also received samples from siblings and other family members and analysed the PK activity of these individuals. In the family, the daughter has two further brothers (Table 8).

The PK K_M data for other family members is also shown in Table 8.

Task 3c

Write your values for the K_M , derived from the Hanes-Woolf calculation, for the Father, Mother and Daughter in the table for your convenience (**zero marks**).

Table 8. K_M [PEP] for subjects in the extended family; the Proband is a person serving as the starting point for the genetic study of a family.

Subject	K_M [PEP]
Daughter (IV-3) (Proband)	
Aunt (III-6)	0.45
Grandfather (II-2)	0.40
Great Grandmother (I-1)	0.40
Mother (III-3)	
Brother (IV-4)	0.48
Brother (IV-5)	0.35
Father (III-4)	
Grandmother (II-1)	0.20
Aunt (III-2)	0.15
Uncle (III-5)	Unknown/Unavailable

- ? Unkown and unavailable
- Unaffected female
- Unaffected male
- Affected female
- Affected Male

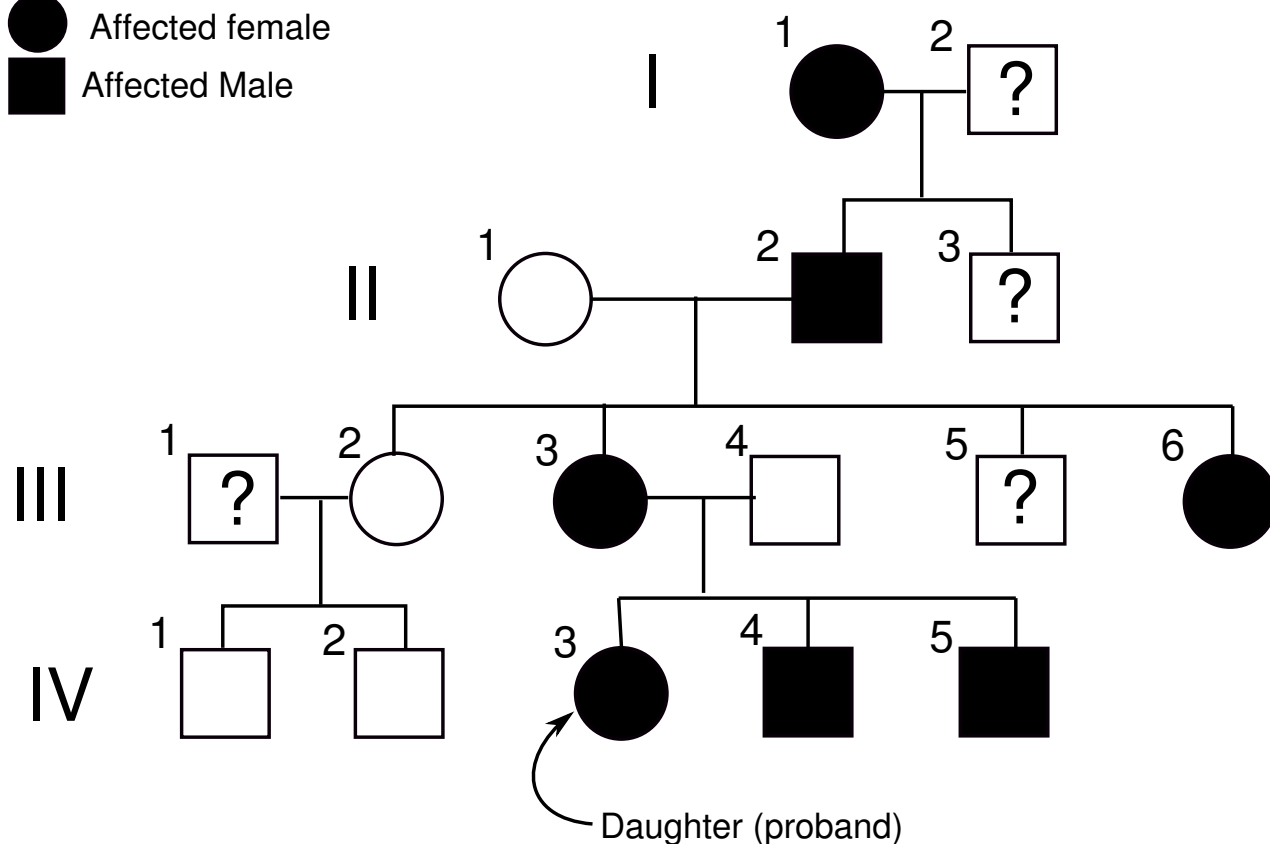


Figure 6. Family Pedigree.

Task 3d (1 mark for the correct answer; you cannot gain a mark for Task 3e unless you have answered Task 3d correctly).

Indicate, by drawing a circle around your choice of either A, B or C, father III-4's genetic PK gene arrangement in terms of:

- A. Simple Heterozygote (Aa_1, Aa_2, Aa_3)
- B. Compound Heterozygote ($a_1a_2; a_1a_3; a_2a_3$)
- C. Homozygous (AA)

Task 3e (1 mark for the correct answer; you can only receive a mark if you have answered Task 3d correctly).

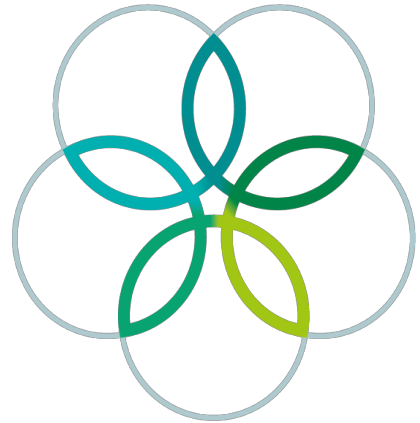
Indicate, by drawing a circle around your choice of either A or B, brother IV-5's genetic PK gene arrangement in terms of:

- A. Simple Heterozygote
- B. Compound Heterozygote

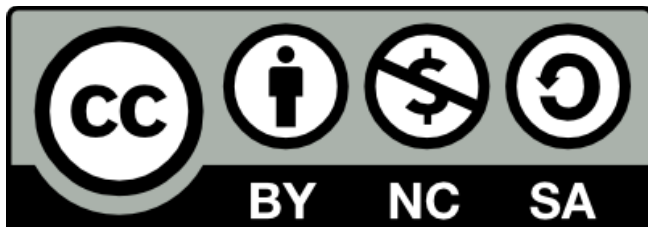
END OF EXAM

INTERNATIONAL
BIOLOGY
OLYMPIAD e. V.

IBO



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Student name:	Student code:	Country:
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28th International Biology Olympiad

July 23-30, 2017
University of Warwick
United Kingdom



International Biology Olympiad

Practical Exam 3

DEVELOPMENTAL PHYSIOLOGY

**The exam will start and
end with a whistle.**

Total points: 100
Duration: 120 minutes

GENERAL INSTRUCTIONS

In this practical test you have **TWO hours** to do **TWO Questions**.

You must perform the tasks in the order given here, Larvae for Q2 will be available from 30 minutes after the start of the examination:

Question 1 - Identifying tissues of a fly larva (45 Marks available)

Tasks 1a and b - Identify the axes of a larva. (7 Marks)

Tasks 1c, d and e - Dissect *Calliphora vicina* larva isolate and identify tissues. (38 Marks)

Question 2 - Physiology of a larval heart (55 Marks available)

Task 2a, b and c - Dissect a *C. vicina* larva to reveal the beating dorsal vessel (larval heart) (10 Marks)

Task 2d, e and f - Devise and perform an experiment to identify the activity of three pharmacological agents acting on the dorsal vessel (45 Marks)

This is a test of fine dissection skills, observation and experimental design.
Good luck!

Important Information:

- Please remember to write your name, your student code and your country in the given boxes.
- Write your answers in this question booklet and place your dissection specimens into the 6 wellled microscope slide. Both items will be collected.
- Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise your Red card immediately.
- During experiments, ensure to handle equipment properly. Any spilled solutions or equipment damaged by you will not be replenished.
- Stop answering and put down your pen immediately when the whistle sounds at the end of the exam.
- Attach your graph paper and your plain paper on to this question booklet with a paper-clip and put in the envelope provided. Ensure to stick your small identification sticker onto the white section of the 6 wellled microscope slide.
- No paper, materials or equipment should be taken out of the laboratory.
- **An English translation of this paper is available upon request.**

MATERIALS AND EQUIPMENT

- 1 x Dissection Stereo Microscope
- 1 x Desk Lamp
- 2 x Pairs Dissecting forceps
- 1 x Pair fine forceps - for moving larvae
- 10 x Dead larvae in a Universal tube. Those have been treated overnight with ethyl acetate (Task 1). More can be made available if required.
- 10 x Larvae treated with a lethal dose of anaesthetic in a glass petri-dish (Task 2). These have been treated for 1 hour with ethyl acetate - these will be provided when requested
- 2 x Plastic dissecting dish filled with a black silicone base
- Approximately 20 minutian pins have been stuck around the edge of each dissecting dish. To move them grip the pins with dissecting forceps close to the the bottom of the pin where it penetrates into the black silicone
- 1 x P1000 pipette plus tips
- 1 x P200 pipette plus tips
- Three 1.5 ml Eppendorfs - labelled A, B, and C, containing Acetylcholine, Adrenaline and Octopamine respectively
- 1.5 ml Eppendorfs for your dilutions
- PBS - Phosphate Buffered Saline - 100 mls
- Gelvitol - mounting media
- Counting clicker - available for beat counts
- 1 x timer
- Microscope slide with six hydrophobic wells marked 1-6 (Do not touch the hydrophobic wells)
- Sheets of paper towel
- 1 x Marker pen
- 1 x Graph paper
- 1 x Plain paper sheet
- 1 x Paper clip
- 1 x Red Flag (dark if colourblind)
- 1 x Green flag (light if colourblind)
- 1 x Waste beaker

QUESTION 1: IDENTIFYING TISSUES OF A FLY LARVA

Introduction:

The Calliphoridae are typical members of the Order Diptera. Larval anatomy, while often different to the adult, retains many of the adult features. In the first set of experiments you will be required to identify a number of tissues in the larval stages of a *C. vicina*.

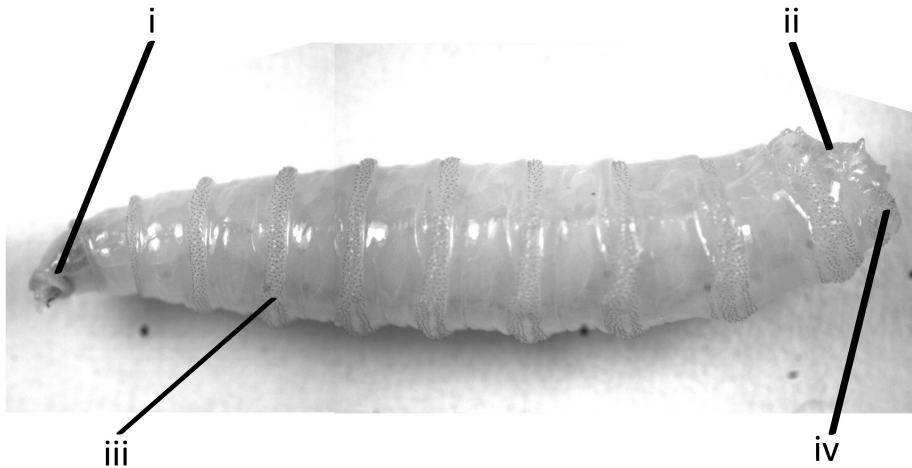


Figure 1. Lateral view of a third-instar larva (a developmental stage) of *Calliphora vicina*, anterior is to the left. i) Pseudocephalon ii) Spiracle field iii) Spinose band iv) Anal division

Anterior to the anal division is the spiracle field where access of air into the tracheal system is gained through a pair of small brown ringed structures called spiracles containing a number of brown slits.

Following dissection using a stereo dissecting microscope, the anatomy of the internal tissues of a larva can be viewed. The structures are represented in Fig.2. Of importance to Experiment 1 is the nervous system, the fat bodies, the alimentary system, the tracheal system and the imaginal discs. The imaginal discs are discrete sheets of epithelia that will form the adult integument following metamorphosis.

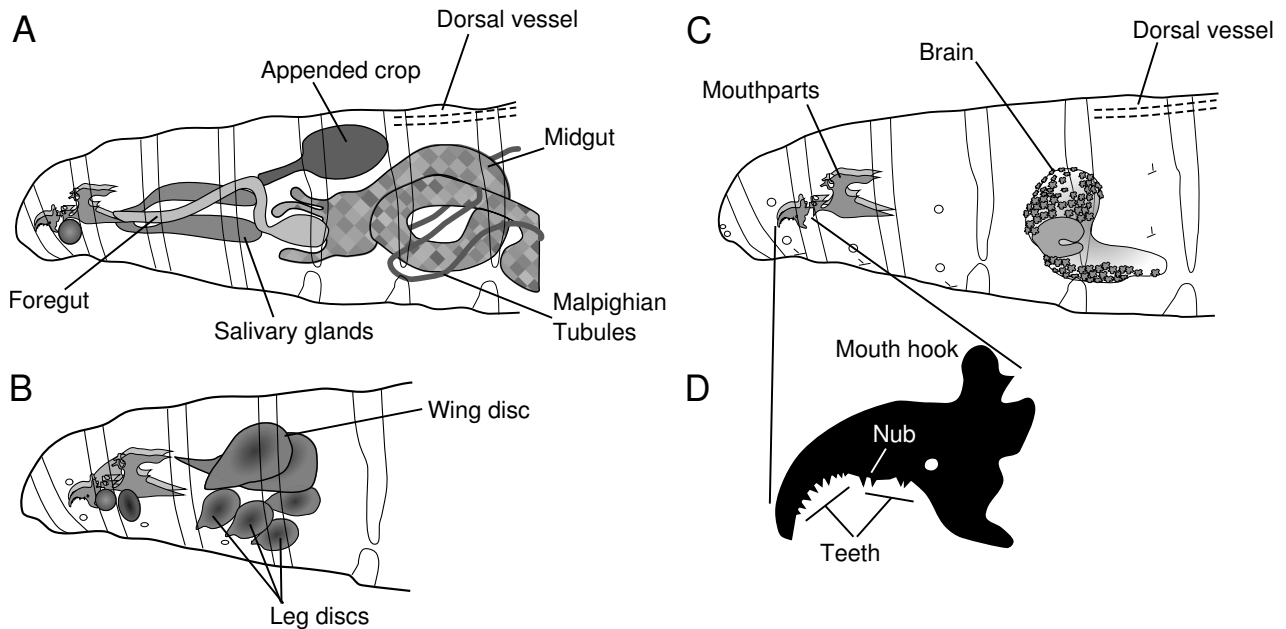


Figure 2. Lateral diagrams of anterior internal structures of a highly derived dipteran larva. *A.* Note position of crop and salivary glands. *B.* Note the position of the wing disc in relation to the mouthparts and brain. *C.* The anterior part of the mouthparts, the “mouth hook”, is decorated by a number of teeth both on the hook and on the nub. These number from 0 - 12 depending on the species and developmental stage e.g. in *D.*

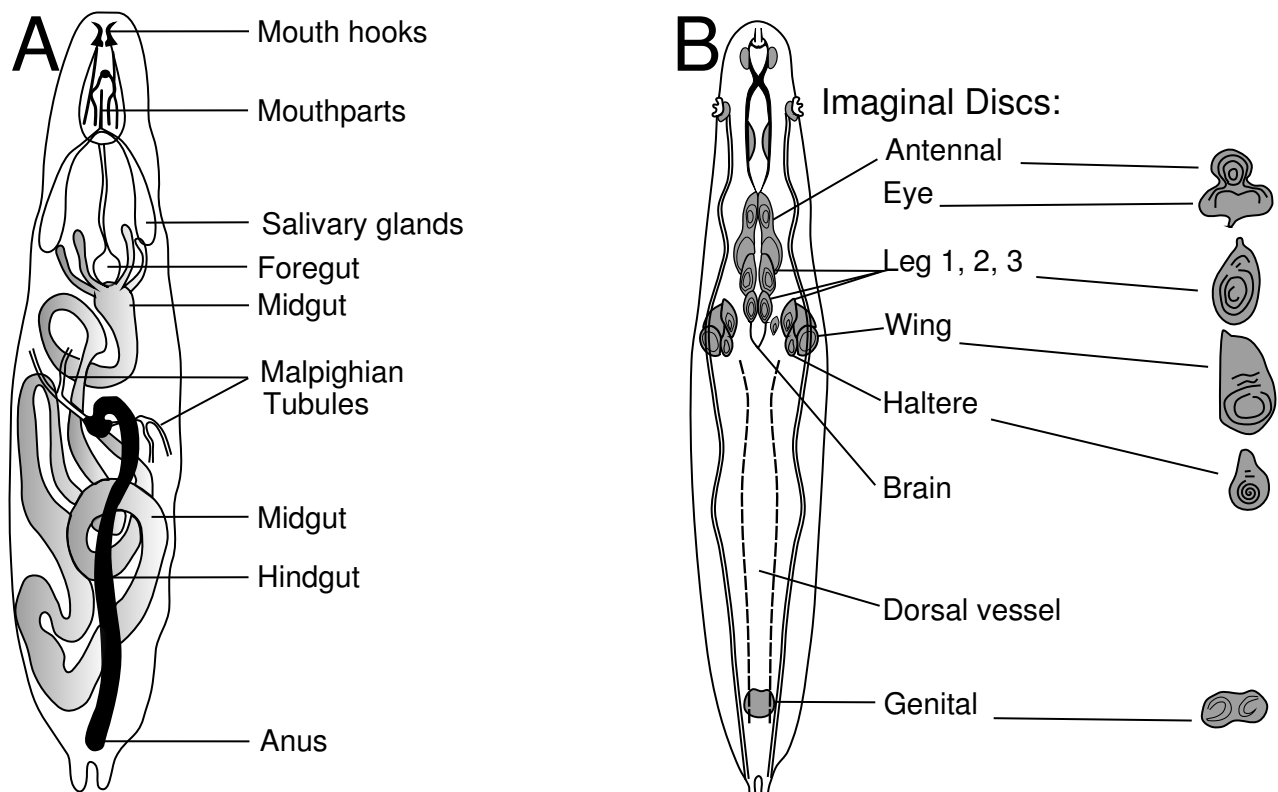


Figure 3. Dorsal representations of a dipteran larvae (not *C. vicina*) *A.* Alimentary system (without crop) *B.* A dorsal representation of the relationship between the brain, major tracheal tubes, dorsal vessel and imaginal discs. Note the relative size and position of the wing disc in the diagrams - in *C. vicina* it is found attached to lateral trachea slightly more anterior to the brain than the diagram here (compare to Fig. 2B).

In the diagrams above, **the fat tissue is not shown**. Fig. 4 shows two partially prepared larvae showing this material - and the natural lack of colour and shading in such preparations.

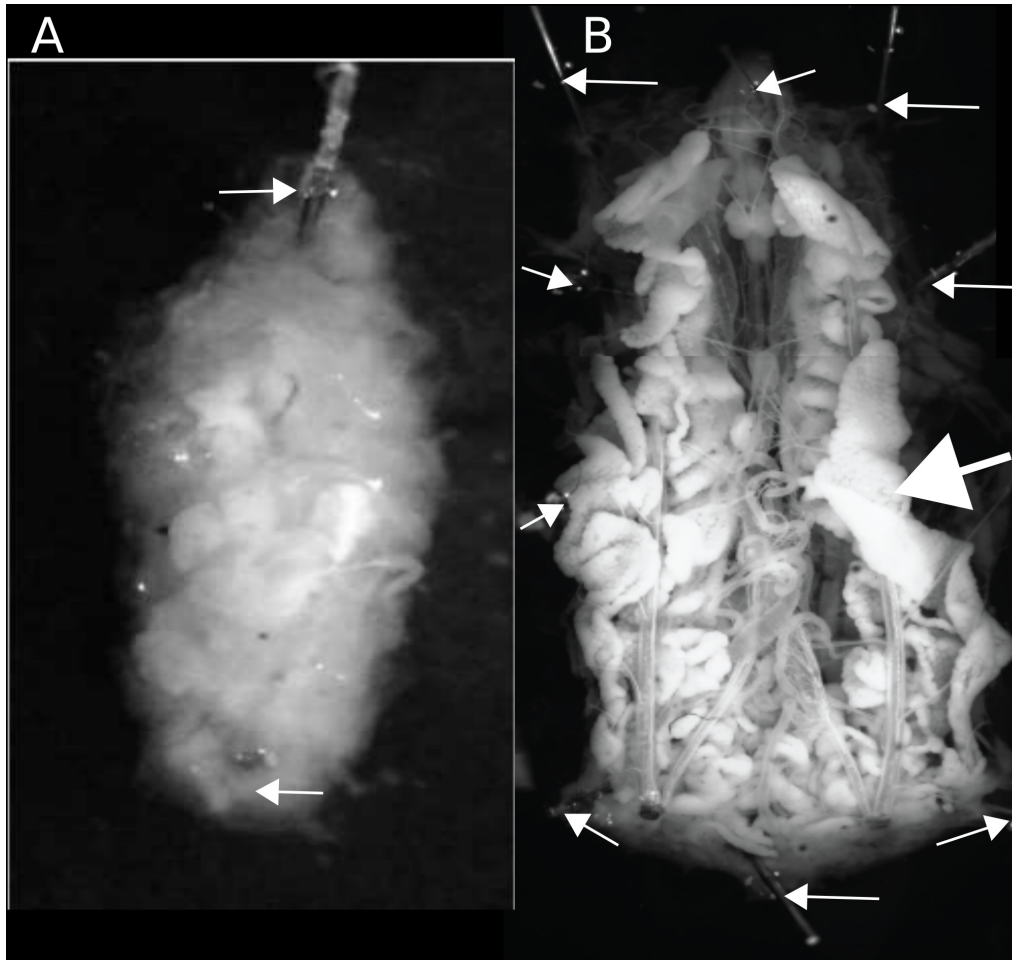


Figure 4. Initial Dissection **A.** Freshly pinned larva, following careful ripping of cuticle. **B.** Filleted and pinned preparation - larger white arrow shows the bright white fat body material making up much of the internal contents. Small white arrows point to minutian pins. Note the thin white/silver trachea throughout the body

Task 1a

Figs 1 and 5 shows the external morphology of a *C. vicina*.

Place a dead larva on the black silicon dissecting petri-dish - observe the structures seen externally. Using both your observations and the above figures, identify the anterior and posterior ends of the larvae as well as the top (dorsal) and bottom (ventral) surfaces.

Task 1b

i) In Fig. 5 place one of the following identification numbers (1, 2, 3 or 4) into the relevant boxes below to denote the orientation of the larvae in the images. You may use numbers more than once. 1 = Ventral anterior; 2 = Ventral posterior; 3 = Dorsal anterior; 4 = Dorsal posterior.

ii) Draw a line from each of the 3 labels (i, ii and iii), in between the images, to the relevant part of either image.

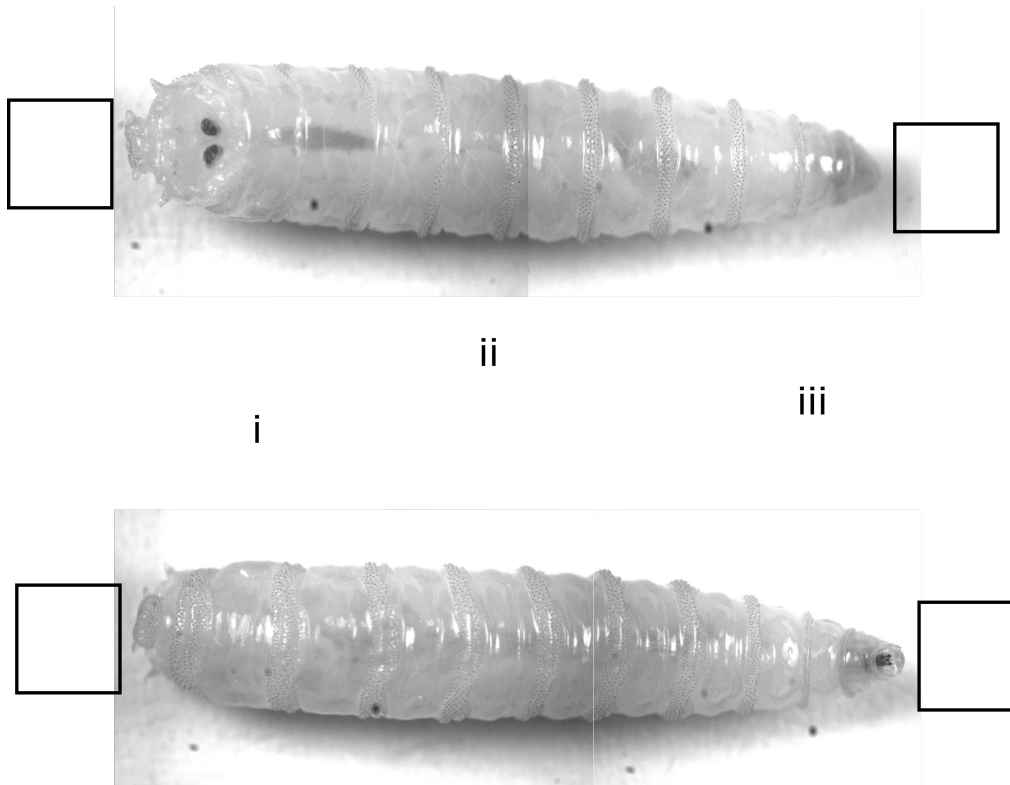


Figure 5. External morphology of *C. vicina*. i) Anal division ii) Pseudocephalon iii) Crop

(7 Marks)

Task 1c + Task 1d

You are required to identify, perform simple measurements and to transfer specific structures to the six-welled microscope slide. You are advised to do all of this whilst looking down the microscope and using your dissecting forceps.

IMPORTANT: The minutian pins should only be moved using forceps whilst looking down the microscope. When pushing them into tissues ensure to hold them closer to the sharp bottom end than the top - they will bend if you do not do this and they will be harder to use. **HINT - ALWAYS** use two pairs of dissecting forceps for the manipulation of the larvae and pins.

BE PROTECTIVE OF YOUR FORCEPS and the preparation. **DO NOT** penetrate the larva too deeply and always tear sideways or up and away from the preparation. It should look similar to Fig. 4A

General Dissection Instructions

1. Using the same larva as in Task 1a, or a new one, put the larva with its dorsal aspect uppermost on your black silicon dish, under the microscope. Using dissecting forceps, pick up a minutian pin and place in the posterior end of the larva through the anal division, just behind the spiracles. With your forceps, gently stretch the larva at the anterior end and insert a pin immediately behind the mouthparts.
2. Pick-up the cuticle between the last two posterior spinose bands and tear apart – the first tear is difficult. This achieved by using the two pairs of dissecting forceps with the larva's posterior end towards you.
3. Using a pipette gently cover the preparation in PBS.
4. Using dissecting forceps rip the cuticle along the mid-line, from the posterior tear to the anterior pin. CAREFULLY place one tip of each forcep just under the cuticle and the other tip on top and gently rip the cuticle by gripping and pulling slightly. Do this in short stages repositioning the forceps after each segment is ripped. Finally turn the dish around and complete any ripping of the cuticle to the posteriorly placed pin.
5. Open out and pin the cuticle flat against the black silicone, producing a “fillet” and exposing the internal structures. Use one pair of forceps to pull back both the cuticle and muscles and the other to manipulate a pin into position. It should look similar to Fig. 4B.
6. Remove excess fat, being careful not to disrupt the brain and gut. Pick up the fat with one forcep, remove and wipe onto tissue or place to one side of the dish.

NOTE: In preparation for this next section place a small drop of gelvitol within each of the hydrophobic wells on your slide. When transferring any tissue from the dissection plate to the microscope slide, aim to pick up the tissue in some fluid so the fluid is held between the tips of the forceps by surface tension. Upon addition to the gelvitol, open the forceps and the tissue will transfer in to the gelvitol.

Task 1d

1. Remove the Appended crop and put at position 1 on your microscope slide. In Table 1, report whether this connects to the alimentary system anteriorly or posteriorly to the brain.
2. Identify the salivary glands, remove one and place at position 2 of your slide. Estimate the ratio of one salivary gland's length to body size length and write this in Table 1.
3. Gently dissect out the brain by removing trachea and breaking the attached nerves and place at position 3 on your microscope. Count the total number of nerves originating dorsally and ventrally from the brain, insert this information in to Table 1.
4. Dissect a posterior spiracle with a small attached section of trachea, place at position 4 on your microscope slide. In Table 1 state the number of slits in one spiracle.
5. Remove the mouthparts. Carefully tease away excess muscle material, free the mouth hooks, separating them from the mouthparts and from each other. Place both mouth hooks into position 5 on the microscope slide. Carefully observe, using transmitted light, to confirm the number and position of teeth on a mouth hook. Record this in Table 1.
6. Find and dissect a wing disc, carefully place into position 6.
7. **IMPORTANT** - You must firmly attach your identification sticker to the white part of the microscope slide. Your slide will be collected and marked at the end of the examination.

Task 1e

You will be scored on the correct tissue in the gelvitol drops and whether it is intact, as well as the answers you give in the table below.

Table 1 Recording of identified larval tissues

Slide Position:	Structure	RECORD YOUR OBSERVATIONS AND ANSWERS IN THIS COLUMN
1	Crop (3 marks)	<p>Is the connection of the crop to the alimentary canal posterior or anterior to the brain? Circle the correct answer.</p> <p style="text-align: center;">ANTERIOR</p> <p>POSTERIOR</p>
2	Salivary glands (4 marks)	What is the approximate ratio of salivary gland length to length of the larval body?
3	Brain (6 marks)	<p>Total number of nerves:</p> <p>Dorsally originating:</p> <p>Ventrally originating:</p>
4	Spiracle and Trachea (7 marks)	Number of slits within a spiracle:
5	Mouth hooks (8 marks)	<p>Total number of teeth:</p> <p>Directly on the nub:</p> <p>Anterior to the nub:</p> <p>Posterior to the nub:</p>
6	Wing disc (10 marks)	Nothing applicable

(Table Total - 38 Marks)

QUESTION 2: PHYSIOLOGICAL RESPONSES OF THE LARVAL HEART.

Introduction

Larvae in the glass petri-dish have been exposed to a lethal dose of anaesthetic. However, the tissues of these animals can, for a short time, still respond to pharmacological agents in a similar way to mammalian systems, with some possible differences. You are required to demonstrate the effects of three pharmacological agents on the beating dorsal vessel: Acetylcholine (A), Adrenaline (B) and Octopamine (C). This organ has evolved in insects to circulate hemolymph through their open circulatory systems. It is equivalent to a mammalian heart. Similar genes are required for development in both insects and mammals. Some mammalian hormones and neurotransmitters can act directly on the insect tissue through homologous receptor proteins.

Task 2a

When you are ready, hold up your green flag and a demonstrator will give you 10 freshly anaesthetised larvae in a glass petridish. Under your dissecting microscope pin a larva with its **ventral** surface uppermost. First place a pin through the anal division. Holding the head/mouthparts, stretch the larva a little without twisting or rotating it and pin just behind the pseudocephalon.

Task 2b

Use the same dissection technique as in Question 1, this time with the ventral surface uppermost. Once you have the fillet, this time CAREFULLY pick-up the gut and Malpighian tubules and pin to the side. Fat can similarly be moved to one side or removed. When picking up these tissues, you will need to carefully break some tiny trachea. The dorsal vessel is a tube running along the dorsal midline (Fig. 1 and Fig. 6). Do not remove the brain or too many trachea, do not touch or damage the dorsal vessel with your forceps directly or indirectly when moving other tissues. All of these may prevent the “heart” from beating. If the PBS wash is cloudy from lipid droplets, then carefully remove and replace the PBS using a P1000 pipette and tips.

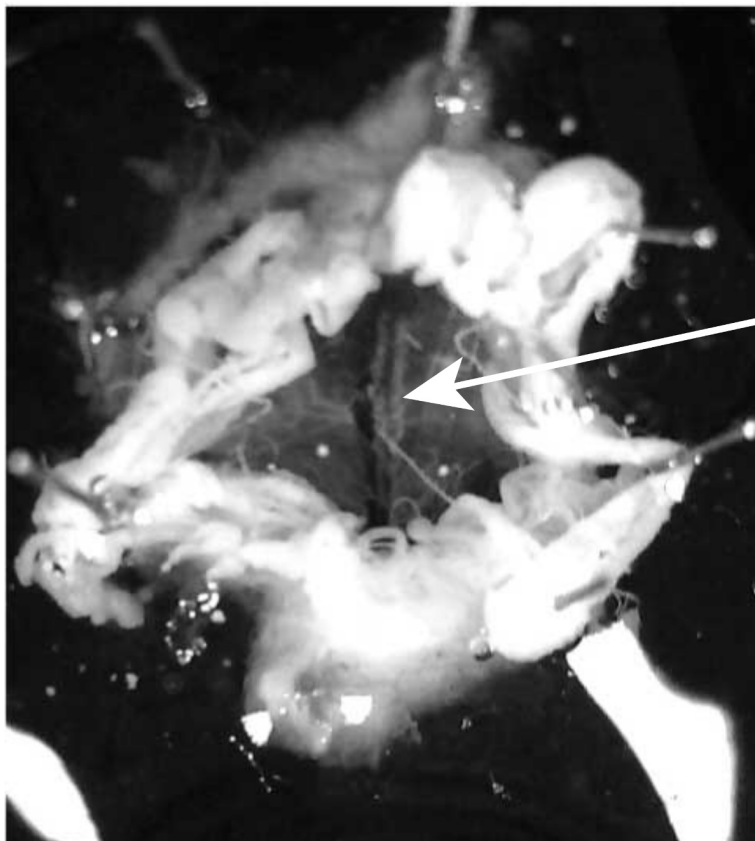


Figure 6. A VENTRAL dissection of a C.vicina larva - following careful moving of the nervous system, gut, trachea and fat, reveals the dorsal vessel (denoted by the arrow)

Task 2c

Observe the dorsal vessel, you should see two parallel lines, if the dorsal vessel is beating it will pulse/move in opposite lateral directions and back together again in synchronisation, this is one heart beat. Once you are satisfied with your preparation, raise your Green flag and a demonstrator will sign on this sheet to confirm that 1) the dorsal vessel has been correctly exposed and 2) it is intact and beating.

Your demonstrator will show you a red flag if the dorsal vessel is not exposed

or

Your demonstrator will show you both a red and a green flag if you have exposed the dorsal vessel, but it is not beating

or

Your demonstrator will show you a green flag if you have successfully exposed the dorsal vessel and it is beating.

You have 10 trials/attempts at this dissection, once you have successfully exposed a beating dorsal vessel and have had this confirmed by a demonstrator (they will write a specific code on your exam paper) you can progress onto Task 2d.

If you are running out of time or larvae or you do not wish to attempt any further dissections then you MUST sign below the table before moving on to Task 2d to finish the examination. Otherwise no credit will be given for Task 2d.

	Demonstrator code
Correctly exposed dorsal vessel NOT Beating	(5 Marks)
Alternatively Correctly exposed dorsal vessel beating	Alternatively (10 Marks)

Once the demonstrator has signed and you wish to progress to the next task, please sign below.

Task 2d

Observation - In mammals heart rate is affected by numerous agents. Neurotransmitters such as acetylcholine decrease the strength (negatively inotropic) and the rate (negatively chronotropic). Alternatively, hormones such as adrenaline increase strength (positively inotropic) and rate (positively chronotropic).

Hypothesis - Such observations lead to the hypotheses that: (H1) Acetylcholine will decrease the beats per minute (BPM) of the dorsal vessel. (H2) Adrenaline will increase the BPM of the dorsal vessel in this species. On to Fig. 7 draw a single-line graph of your **expected** results showing the effects of A, B and W (PBS Wash), applied to a **single** preparation. Ensure to indicate the changes to bpm of the dorsal vessel during the time course of the experiment.

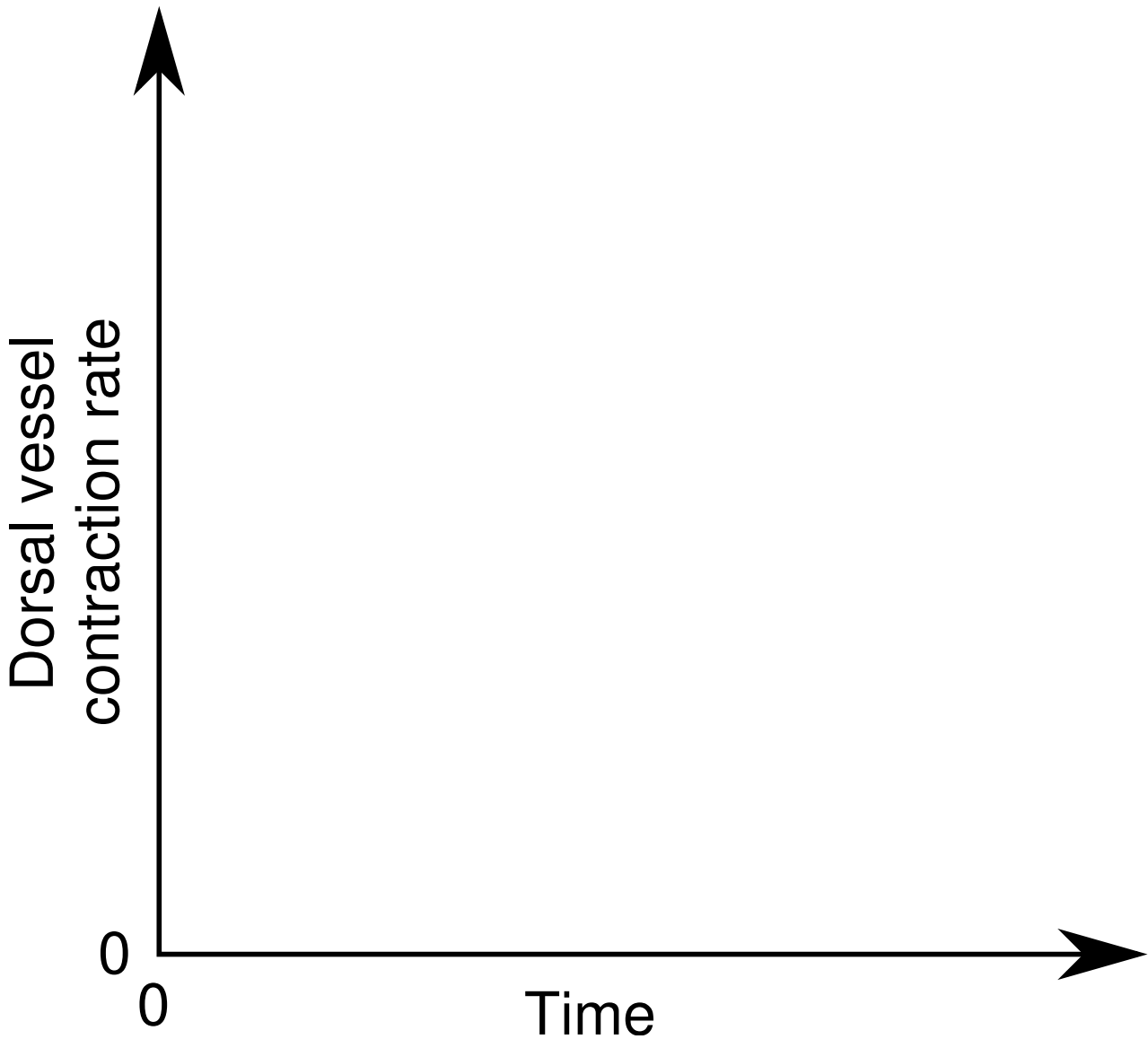


Figure 7. Line graph of expected results for addition of Acetylcholine (A) and Adrenaline (B) and PBS wash (W) to the *C.vicina* dorsal vessel

(7 Marks)

1. You are required to design and perform an experiment to test the hypotheses H1 and H2 and to discover how the insect hormone, Octopamine (C) acts on the dorsal vessel isolated in Task 2b and confirmed in Task 2c.
2. The block below represents the duration of the ideal experiment you would conduct on your single larva preparation. Divide up this block into sections to represent the order you would apply the respective solutions (A, B and C) and PBS wash (W) on **one** larvae preparation from Task 2c. Write the appropriate letter into each division, the first two have been done for you.
3. When designing your experiment you need to consider and effectively represent, washing of the tissue and repetitions.

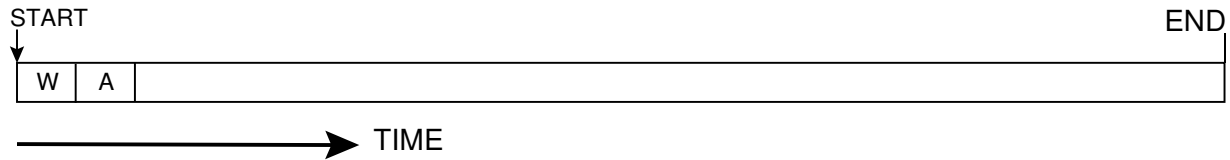


Figure 8. Experimental design. Planned addition of PBS washes (*W*) and agents

(12 Marks)

Task 2e

1. Cover your preparation with fresh PBS (*W*) and record the baseline beats per minute (bpm) in Table 2.
2. Three eppendorfs marked A, B, and C contain stocks of:
 - A = 2×10^{-2} M Acetylcholine
 - B = 2×10^{-2} M Adrenaline
 - C = 2×10^{-2} M Octopamine

100 ml PBS is also provided in a medical flat bottle

Just before use, dilute A, B and C in PBS to their working concentrations of 5×10^{-3} M in 1 mL in Eppendorf tubes. For each agent record volumes for dilutions in Table 2 in μL .

3. Considering your ideal experimental design (Fig. 8), evaluate the effects of A, B, C and W on a single dissected preparation taking into account the time you have left. Record your raw data and average bpm in Table 2 for the dorsal vessel at rest and for the effect of each of the agents A, B and C.
4. Based on your data select the relevant response of bpm in the table e.g Increase, No change within 10% or Decrease in the table
5. From your data indicate whether the receptor for each agent is demonstrating activity or not by writing "1" or "0" respectively in the relevant column of row 4.

Table 2. BPM of resting tissue, effect of agents A, B and C and identification of agents.

Solutions	W	A	B	C
Dilution volumes (μL)	Not required	Stock: PBS:	Stock: PBS:	Stock: PBS:
Record your raw data counts here				
Average bpm	Average =	Average =	Average =	Average =
Select one of the following responses.	Increase No Change within 10% Decrease	Increase No Change within 10% Decrease	Increase No Change within 10% Decrease	Increase No Change within 10% Decrease
Identify agent receptor activity 1 = there is activity 0 = there is no activity				

(12 Marks)

Task 2f

1. On the Graph paper provided, plot a suitable graph of your data over the time course of your experiment. Remember this should represent data applied to a single preparation.

(12 Marks)

2. For this experiment H1 was that the *C. vicina* dorsal vessel will respond to acetylcholine negatively chronotropically and H2 was that adrenaline acts positively chronotropically, as would a mammalian heart.
3. You are now required to accept or reject these hypotheses, you **must** base your decision on the data that you have generated.
Circle your decision below:

H1 ACCEPT REJECT

H2 ACCEPT REJECT

(2 Marks)

END OF EXAM