Student name:	Student code:	Country:

28th International Biology Olympiad

July 23-30, 2017
University of Warwick
United Kingdom

Practical Exam 3

DEVELOPMENTAL PHYSIOLOGY

The exam will start and end with a whistle.

Total points: 100
Duration: 120 minutes

GENERAL INSTRUCTIONS
答題說明

General Instructions(答題說明)

In this practical test you have **TWO hours** to do **TWO Questions**.
(本部分共兩題，請在兩小時內答完)

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:
(請務必按照指定順序答題，第二題之幼蟲將在考試開始後30 分鐘內發給應考同學)

Question 1 – Identifying tissues of a fly larva (45 Marks available)(問題1、辨認及標示出蠅類幼蟲之各項組織) (45分)

Tasks 1a and b – Identify the axes of a larva. (7 Marks)
問題1a及b：請標示出幼蟲之體軸 (7分)

Tasks 1c, d and e – Dissect *Calliphora vicina* larva isolate and identify tissues. (38 Marks)
問題1c、d及e：請小心地將*Calliphora vicina*幼蟲的各項組織分離並清楚標示 (38分)

Question 2 – Physiology of a larval heart (55 Marks available)
(問題2、幼蟲心臟生理學觀察) (55分)

Task 2a, b and c – Dissect a *C. vicina* larva to reveal the beating dorsal vessel (larval heart) (10 Marks)
問題2a,b 及c：請解剖*C. vicina*之幼蟲以清楚顯示跳動中之背血管(即此幼蟲心臟)(10分)

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)
問題2d,e 及f：請設計並操作一個實驗以確認三種不同藥物對幼蟲背血管之活性及作用(45分)

This is a test of fine dissection skills, observation and experimental design.
Good luck!
本實驗主要測試各位是否具精細解剖技巧，觀察能力及實驗設計。
加油，祝好運!

Important Information:
重要信息：
Please remember to write your name, your student code and your country in the given boxes.
請記住在所提供的框格中寫下您的姓名，學生代碼和國家。
Write your answers in this question booklet and place your dissection specimens into the 6 welled microscope slide. Both items will be collected.
在本試卷紙上寫下你的答案，並將你的解剖標本放入一個六孔顯微載玻片上，此兩項將會被收回。
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.
先確定您已收到所列出的所有材料和設備。如果有任何這些項目遺漏，請立即舉起您的紅卡。
During experiments, ensure to handle equipment properly. Any spilled solutions or equipment damaged by you will not be replenished.在實驗過程中，確保妥善處理設備。任何溢出的溶液或您所損壞的設備將不會被補充 。
Stop answering and put down your pen immediately when the whistle sounds at the end of the exam.
在考試結束時口哨聲響起時，請停止回答並放下筆。
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 welled microscope slide.
將你的作圖紙及白紙用迴紋針夾在試卷紙上，放入提供的信封中。確定將你的小的識別貼紙貼在6孔顯微載玻片的白色部分。
No paper, materials or equipment should be taken out of the laboratory.
不得將紙張，材料或設備攜出實驗室。
An English translation of this paper is available upon request.
本文的英文翻譯依您的要求提供。

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材料與設備

Materials and Equipment (材料與設備)

- 1 x Dissection Stereo Microscope
  一台解剖立體顯微鏡
- 1 x Desk Lamp
  一盞桌燈
- 2 x Pairs Dissecting forceps
  兩把解剖鑷子
- 1 x Pair fine forceps - for moving larvae
  一把精細鑷子
- 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.
  十隻已死之幼蟲(供任務1使用〉已利用ethyl acetate藥物處理整晚，如有需要，有多的樣本可供索取
- 10 x Larvae treated with a lethal dose of anaesthetic in a glass petri-dish (Task 2). These have been treated for I hour with ethyl acetate – these will be provided when requested
  十隻以致死劑量之麻醉劑處理之幼蟲(供任務2使用)。已利用ethyl acetate處理一小時，如有需要有多的樣本可供索取
- 2 x Plastic dissecting dish filled with a black silicone base
  兩個解剖用黑色矽膠的培養皿
- 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
  在培養皿周圍各插入約二十根極細微小針，需用針時，請用鑷子夾住針下方與矽膠接觸處拔出使用。
- 1 x P1000 pipette plus tips
  一支附有吸取尖的微量吸管〈1毫升〉
- 1 x P200 pipette plus tips
  一支附有吸取尖的微量吸管〈 0.2毫升〉
- Three 1.5 ml Eppendorfs - labelled A, B, and C, containing Acetylcholine, Adrenaline and Octopamine respectively
  標示A、B、C的三個1.5毫升的微量離心管，內含液體分別為乙醯膽鹼、腎上腺素、八酚胺。
- 1.5 ml Eppendorfs for your dilutions
  一個稀釋用的1.5毫升的微量離心管
- PBS – Phosphate Buffered Saline – 100 mls
  100毫升的磷酸鹽緩衝溶液
- Gelvitol
  用以包埋之溶液
- Counting clicker
  計數器:用以計算心跳數
- 1 x timer
  一個計時器
- Microscope slide with six hydrophobic wells marked 1-6 (Do not touch the hydrophobic wells)
  一片具有編號1到6的6孔厭水性載玻片〈請勿碰觸厭水圈〉
- Sheets of paper towel
  紙巾
- 1 x Marker pen
  一支麥克筆
- 1 x Graph paper
  一張圖紙
- 1 x Plain paper sheet
  一張白紙
- 1 x Paper clip
  一個迴紋針
- 1 x Red Flag (dark if colourblind)
  一支紅旗〈如色盲者提供深色旗〉
- 1 x Green flag (light if colourblind)
  一支綠旗〈如色盲者提供淺色旗〉
- 1 x Waste beaker
  一個盛裝廢液之燒杯

QUESTION 1: IDENTIFYING TISSUES OF A FLY LARVA
問題一、辨認蠅類幼蟲之組織

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 blow-fly species.

Calliphoridae為典型的雙翅目成員。其幼蟲之解剖構造雖然經常與成蟲不同，但仍有許多構造與成蟲類似。在本實驗中，你必須辨認*C. vicina*.幼蟲體內之多種組織

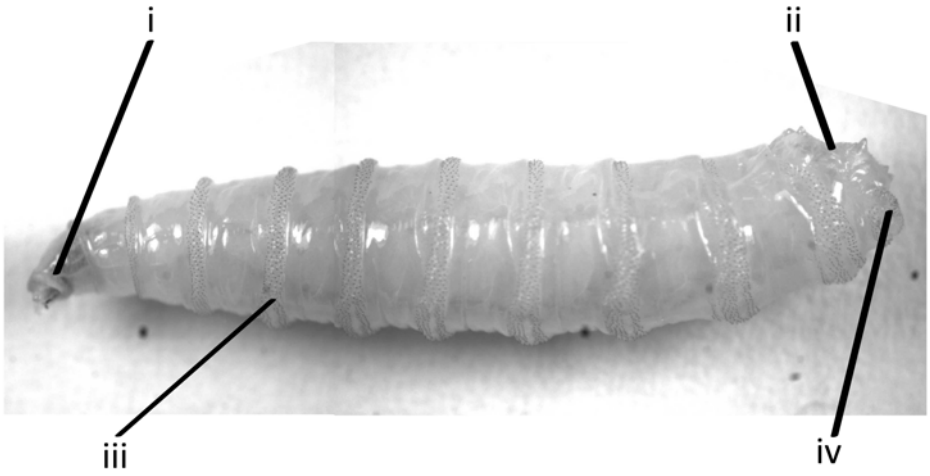

Figure 1. Lateral view of a third-instar larva (a developmental stage) of Calliphora vicina, anterior is to the left. *i) Pseudocephalon ii) Spiracle field iii) Spinose band iv) Anal division*
*圖1.**Calliphora vicina**三齡幼蟲(發育期之一)之側面圖，幼蟲前端在左邊。 i)Pseudocephalon偽頭部。 ii)氣孔部位(spiracle field)。 iii)含刺體節(spinose band)。 iv)肛門區(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.
在肛門區的前方為氣孔部位(spiracle field)，此處為空氣進入氣管系統之主要部位，由一對棕色之環狀構造構成，稱為氣孔，其上具有一定數目之棕色裂隙。
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.sheets of epithelia that will form the adult integument following metamorphosis.
本實驗利用立體解剖顯微鏡，以清楚辨識幼蟲之內部結構。相關結構呈現在圖2。實驗1之重點主要在神經系統，脂肪體，消化系統，氣管系統及器官芽(imaginal discs)。器官芽為不連續之上皮組織，在幼蟲變態後可形成成蟲的表皮組織。

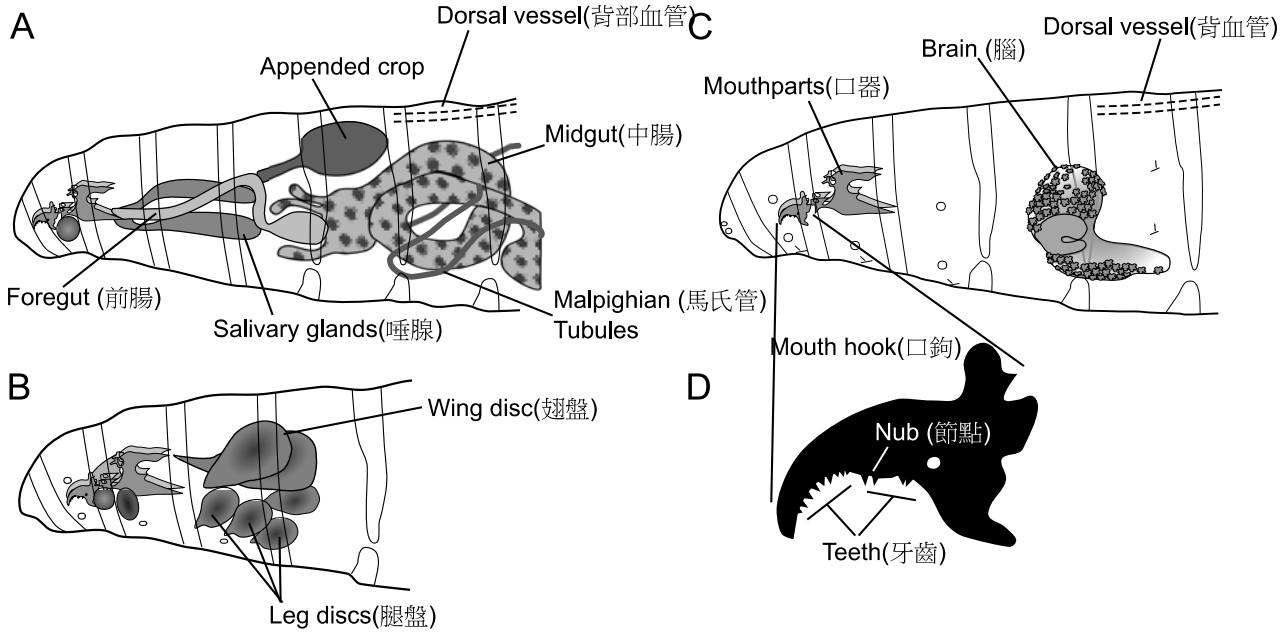

Figure 2.Lateral diagrams of anterior internal structures of a highly derived dipteran larva. ***A.*** *Note position of crop and salivary glands.* ***B****. Note the position of the wing disc in relation to the mouthparts and brain.* ***C.*** *The anterior part of the mouthparts, the "mouth hook", is 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.***

圖2：高度演化之雙翅目幼蟲前方內部構造之側面觀察圖*。A.嗉囊及唾腺之標示位置 B.翅盤與口器及腦的相關位置 C.口器的前端稱為口鉤，口鉤及其後端節點上皆有牙齒，視種類及物種發育階段而異，其數量在0到12之間，如D圖。*

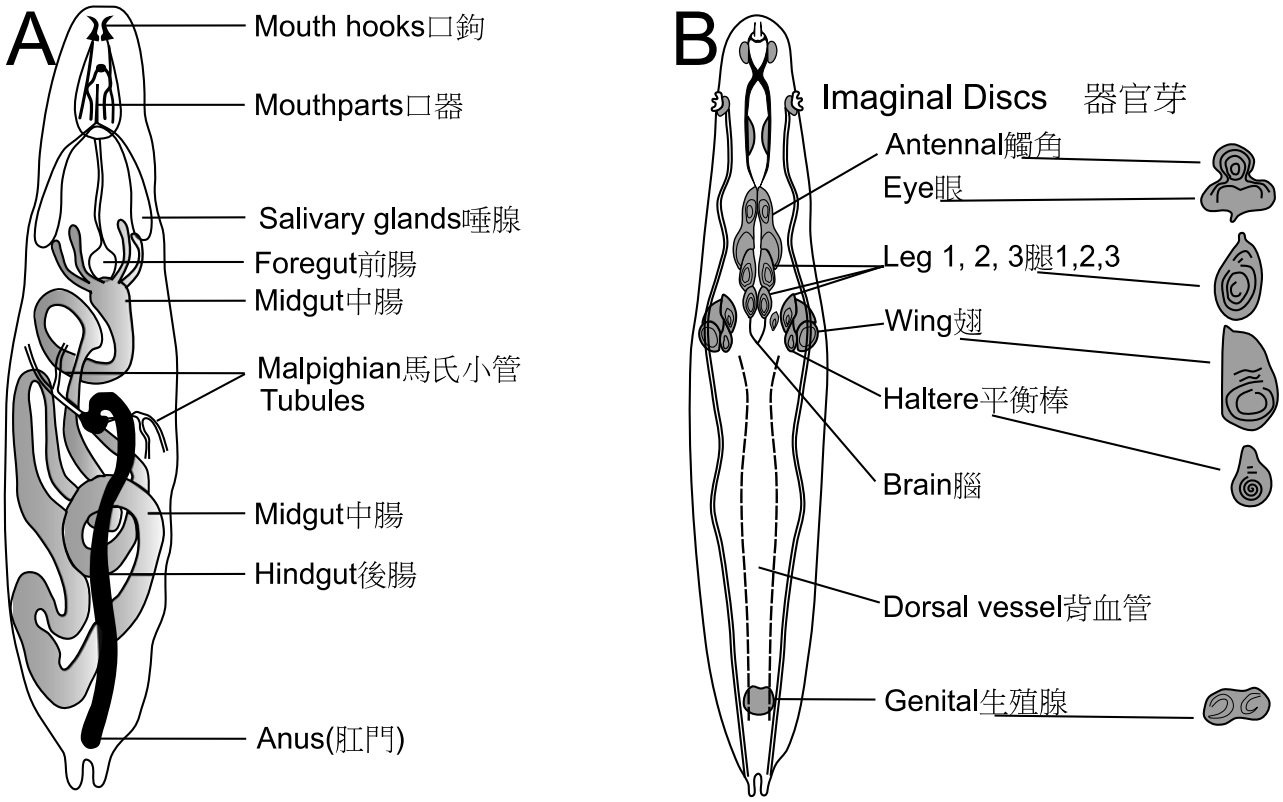

Figure 3. Dorsal representations of a dipteran larvae *(not C.vicina)* ***A****. Alimentary system (without crop)* ***B****. A dorsal representation of the relationship between the brain, major tracheal tubes, dorsal vessel and imaginal discs. Note the relative size and position of the wing disc in the 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).*
圖3、雙翅目幼蟲之背部觀察圖〈並非C.vicina〉。A去除嗉囊之消化系統。B自背部觀察腦，主要氣管系統，背血管及器官芽之關係圖。請注意翅盤在此圖中之相對大小及位置-在C.vicina之翅盤會與側面氣管連結並比此圖更向前靠近腦的位置〈與圖2B比較〉

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.
上圖並無顯示脂肪組織。圖4顯示兩隻部分處理過之幼蟲，顯示處理後脂肪本質上不具顏色及層次

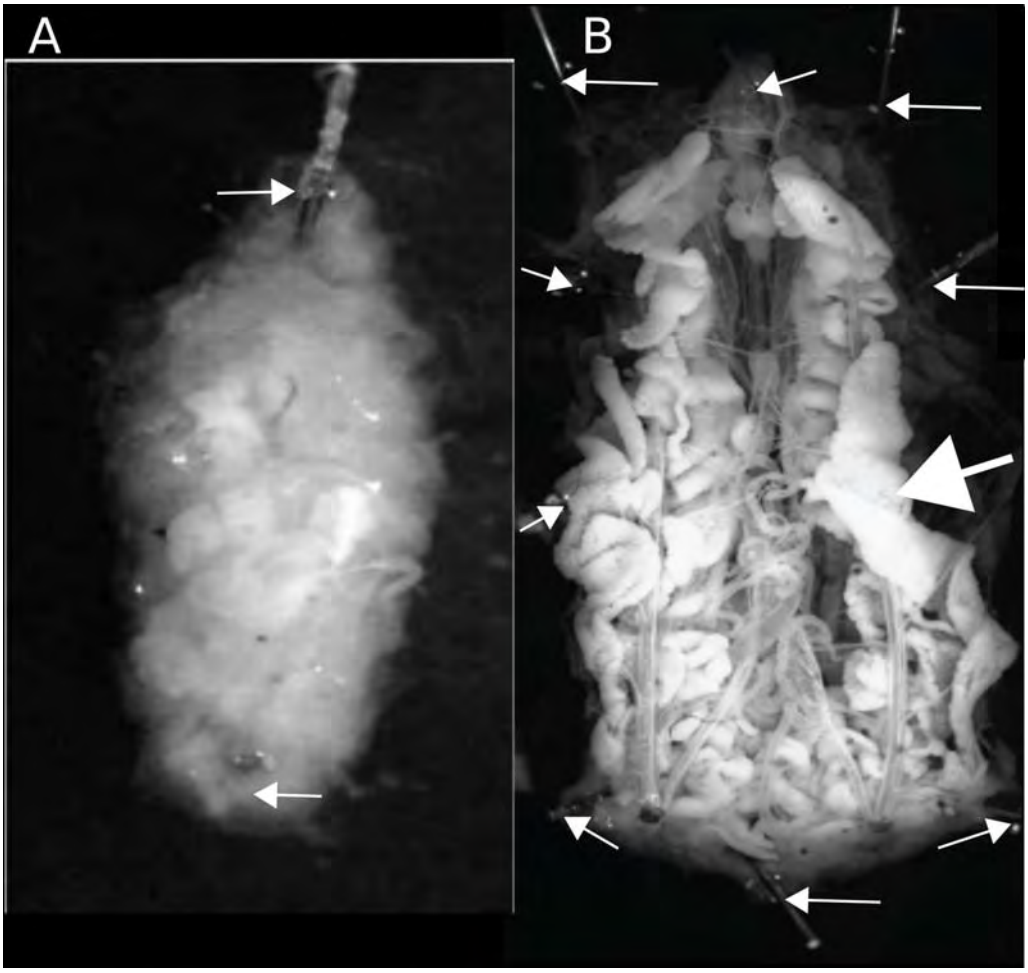

Figure 4*. *Initial Dissection A. *Freshly pinned larva, following careful ripping of cuticle.* ***B.*** *Filleted and pinned preparation – larger white arrow shows the bright white fat body material making up much of the internal contents. Small white arrows point to minutian pins.Note the thin white/silver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

圖4最初之解剖圖 A.新鮮製備已經仔細剝除外皮之幼蟲標本。 B. 剝製並由針固定的標本– 較大的白色箭頭表示明亮的白色脂肪體布滿著大部分之內臟空間。 小白色箭頭指示極細微小針。請注意分布於全身之白色/銀色氣管

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.

任務1a
圖1及圖5顯示*C.vicina*.之外部形態。
將一隻已死之幼蟲放在所提供之黑色培養皿上。仔細觀察外部構造，請利用你的觀察力及上面的圖，辨認出幼蟲之前端、後端、頂部〈背部〉及底部〈腹部〉。

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.

任務1b

i) 在圖5中，請在相關空格中填入下列號碼(1,2,3,4)以確認幼蟲之方向，號碼可重複使用。1表示腹部前方；2表示腹部後方；3表示背部前方；4表示背部後方。

ii）自圖中找出代表i,ii,iii之構造，並在圖上用直線清楚標示位置

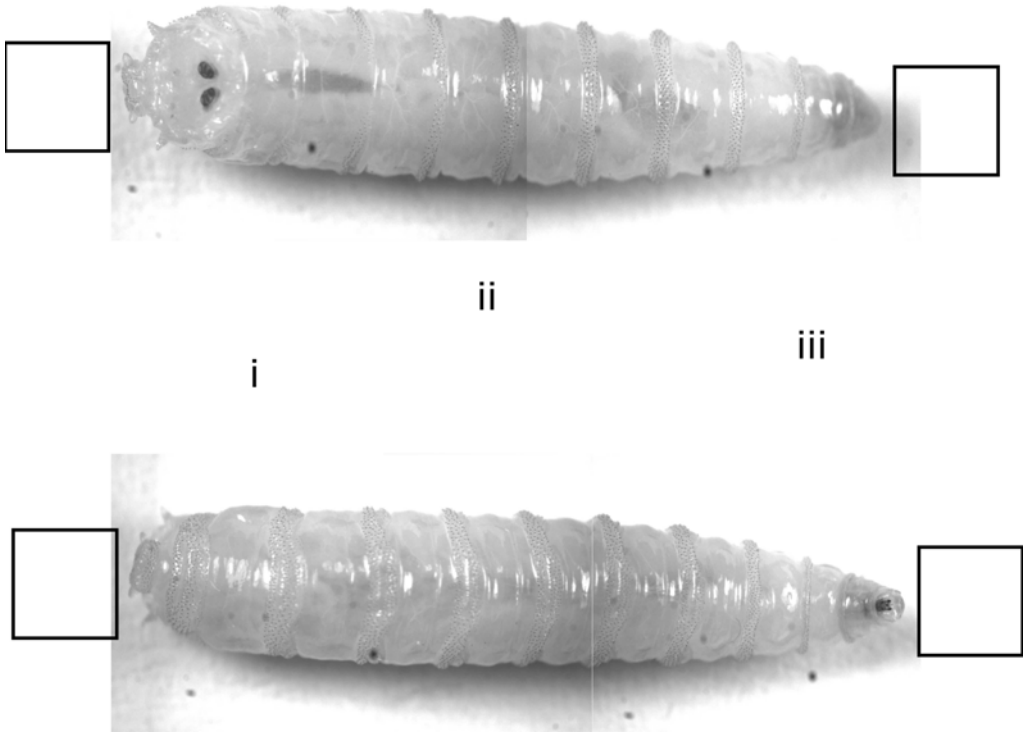

Figure 5. External morphology of C. vicina*. i) Anal division ii) Pseudocephalon iii) Crop*
*圖5、**C. vicina**之外部構造(i)肛門(ii)偽頭部(iii)嗉囊*

(7 Marks)7分

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.

任務1c及1d

在本實驗中，你必須辨識並簡單量測所取出並放置於六孔顯微玻片上之組織。建議你在解剖顯微鏡下及利用所提供之解剖鑷子進行此實驗

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.

重要!

極微細的小針只可在顯微鏡下用鑷子移動，如將小針推向組織時要先確認已夾緊其較尖銳之下半部而非頂端，如果不這麼做，他們會變彎曲，更難操作。

提示！一定要使用兩把解剖鑷子來操作幼蟲及小針

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
保護好你的鑷子及實驗標本。千萬不要刺穿幼蟲太深，一定要向兩邊或向上挑開幼蟲組織。應與圖4A相似。

General Dissection Instructions 解剖通則

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
在顯微鏡下，將實驗1a之幼蟲(或拿隻新的也可以)，背部朝上置於黑色矽膠培養皿中。用解剖鑷子，小心取出極微細的小針並將其穿過幼蟲緊鄰氣孔後端的肛門區。利用鑷子，小心地從前端拉直幼蟲，在口器後端插入一小針。
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
將幼蟲最後兩含刺體節間之表皮提起並撕開，第一次撕最困難，請小心。建議你把幼蟲之後端移向你並利用兩把解剖鑷子來操作。
3. Using a pipette gently cover the preparation in PBS.
以微量吸管吸取PBS蓋過幼蟲樣本
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.
使用解剖鑷子沿中線從後往前撕開角質層。 **千萬小心!** 將每個鑷子的一個尖端放在角質層之下，將鑷子另一個尖端放在頂部，並通過夾住組織和輕拉的動作輕輕撕開角質層。分小段進行， 撕開每段後，重新放好鑷子。 最後旋轉培養皿，並自前往後打開角質層到標識尾端之小針。
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.
打開角質層後將其平鋪，並以小針將角質層固定在黑色矽膠培養皿上，產生一個剝皮後之蟲體，並呈現所有內部構造。用一隻鑷子拉回角質層及肌肉，用另一隻鑷子定位解剖針。其結果應與圖4B相似。
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.
移除多餘脂肪，小心不要破壞腦及腸。利用鑷子的一邊輕輕移除脂肪，用紙擦拭或放置到培養皿之一端。

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.
進行下列實驗時，請將一小滴gelvitol放入載玻片上之每一厭水小圈內。當你將組織從解剖盤中移到載玻片時，盡量帶有一些液體，因為表面張力關係，液體會留在鑷子兩端。加入gelvitol時，輕輕張開鑷子，組織就會輕輕滑入gelvitol中。

Task 1d
任務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
將嗉囊移除並放入顯微玻片上編號1之位置中。請在表1中寫出嗉囊是否在腦的前端或後端與消化系統連結
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
辨識出幼蟲之唾腺並取其一置入於顯微玻片位置2中。在表1中寫出你估算一個唾腺的長度與體長之比例。
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 from the brain, insert this information in to Table 1.
小心地將腦移除：切除所連結之神經及氣管系統，將腦分離出來放入顯微玻片位置3中。請算出所有自腦中發出之神經數目寫入表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.
將後方氣孔及其所連結之小部分氣管分離出來後放入顯微鏡玻片之位置4。在表1中寫出一個氣孔上的裂縫數目。
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
移除口器。仔細將口器旁多餘的肌肉剔除，將口鉤與口器彼此分開。 將兩個口鉤放入顯微鏡玻片之位置5中。利用穿透之光線仔細觀察並確認一個口鉤上的牙齒數及位置並記錄在表1中。
6. Find and dissect a wing disc, carefully place into position 6
找出並分離出幼蟲之翅盤，將其置於顯微鏡玻片之位置6中
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.
重要提醒**---**你必須將識別貼紙牢貼在顯微鏡載玻片之白色部分。你的玻片會在實驗結束時被收集並打分數，所以務必貼牢。

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.

任務1e

本部分評分依據為你是否分離出正確之組織置於gelvitol中；組織完整性及表1的答案。

Table 1 Recording of identified larval tissues (表1幼蟲組織辨識紀錄表)

Slide Position:	**Structure**	**RECORD YOUR OBSERVATIONS AND ANSWERS IN THIS COLUMN**
1	Crop 嗉囊 〈3分〉	Is the connection of the crop to the alimentary canal posterior or anterior to the brain? Circle the correct answer. 嗉囊在腦的前端或後端與消化系統相連？請圈出正確答案。 ANTERIOR 前端 POSTERIOR後端
2	Salivary glands 唾腺 〈4分〉	What is the approximate ratio of salivary gland length to length of the larval body? 所預測唾線長度與體長之比例為何？
3	Brain 腦 〈6分〉	Total number of nerves:所有神經數目 Dorsally originating:自背側發出之神經數目 Ventrally originating: 自腹側發出之神經數目
4	Spiracle and Trachea 氣孔及氣管 〈7分〉	Number of slits within a spiracle: 每個氣孔之裂縫數
5	Mouth hooks 口鉤 〈8分〉	Total number of teeth: 牙齒總數 Directly on the nub: 節點上之牙齒數 Anterior to the nub: 節點前之牙齒數 Posterior to the nub: 節點後之牙齒數
6	Wing disc 翅盤 〈10分〉	Nothing applicable 無須作答

(表1總分-38 分)

QUESTION 2: PHYSIOLOGICAL RESPONSES OF THE LARVAL HEART.
問題二:幼蟲心臟之生理反應

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.

介紹
在玻璃培養皿中的幼蟲已暴露於致死劑量的麻醉劑，然而其組織在短時間內仍能以類似哺乳動物系統的方式對藥物產生反應，但仍可能會有些差異。你必須證明下列三種藥物：乙醯膽鹼（A）、腎上腺素（B）、八酚胺（C）對跳動中背血管的作用。背血管相當於哺乳動物的心臟，是能讓昆蟲的血淋巴在其開放式循環系統循環的器官。昆蟲和哺乳動物的發育都需要類似的基因。某些哺乳動物的激素和神經傳導物可以通過同源的受體蛋白直接作用於昆蟲組織。

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

任務2a
當你預備好的時候，舉起綠色旗，助教才會給你10隻剛麻醉好的幼蟲放在玻璃皿中。在解剖顯微鏡下，將一隻幼蟲腹部向上釘好。先以針釘住肛門部分，抓住頭部/口器部位來輕輕拉直幼蟲，不要扭曲或旋轉它，並藉由鑷子用微細針釘住在偽頭部的後方。

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.

任務2b
此實驗之解剖技巧同問題1，只是這次是將幼蟲腹部向上放在黑色培養皿中。剖好幼蟲後，這次要很小心地取出腸道和馬氏管並將其用細針固定在旁邊。可以將脂肪移到一邊或去除。取出這些組織時，需要小心地弄斷一些小氣管。背血管是沿著背部中線延伸的血管（圖1和圖6）。不要去除腦或太多氣管，在移動其他組織時，不要直接或間接用鑷子觸到或傷到背血管。所有這些都可能阻止“心臟”跳動。因為這些都可能阻礙“心臟”跳動。如果有油脂小滴使PBS渾濁，用P1000微量吸管與吸管尖將其小心吸出並更換PBS。

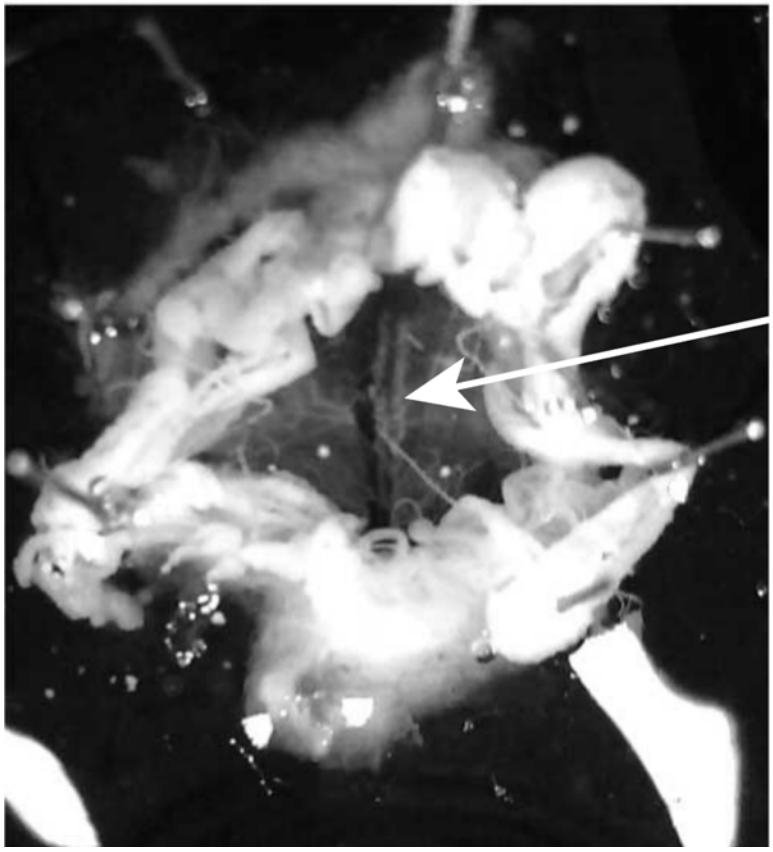

Figure 6. A VENTRAL dissection of a C.vicina larva – following careful moving of the nervous system, gut, trachea and fat, reveals the dorsal vessel (denoted by the arrow)

圖6. C. vicina幼蟲的腹面解剖 - 小心地移開神經系統、腸道、氣管和脂肪後，顯示其背血管（箭頭所示）

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.

任務2c
觀察背血管，應可看到兩條平行線，如果背血管正在跳動，會將液體朝相反方向搏動/移動，然後再同步一起回來完成一次心跳。你若認為已解剖好了就舉綠色旗，請助教在表上簽名，以確認(1)背血管已正確指出及(2)背血管完好無損並在跳動。
如果背部血管未被正確解剖出來，助教會給你紅色旗
或
如果你解剖出背血管，但卻不會跳動，助教會給你紅色和綠色旗
或
如果你成功解剖出背血管並且正在跳動，助教會給你綠色旗。

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.

這個解剖實驗你可有10次試驗/嘗試，一旦剖露出一個跳動的背血管並已向助教證明（他們會在試紙上寫一個特定代碼），就直接跳到任務2d。

如果時間不夠，你不希望再做解剖，或10隻蟲體用完，不能再做，你必須先在表格下方登錄，再到任務2d完成考試。否則任務2d不會給你任何分數。

	Demonstrator code （助教給代碼）
Correctly exposed the dorsal vessel NOT Beating 正確顯示背血管，但未跳動。	（5分）
Alternatively Correctly exposed dorsal vessel beating 正確顯示正在跳動的背血管	（10分）

Once the demonstrator has signed and you wish to progress to the next task, please sign below.
當助教已簽名認可，而且你希望進入下個任務，請在下方簽名。

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.

任務2d
觀察：哺乳動物的心跳速率會受到多種藥物的影響。神經傳導物如乙醯膽鹼會降低心肌收縮強度（降低心肌收縮力）和降低心跳速率（抑制心跳）。而激素如腎上腺素會增加心肌收縮強度（增強心肌收縮力）和速率（促進心跳）。
假說：由這些觀察結果導出以下的假說：（H1）乙醯膽鹼會降低每分鐘背血管的搏動次數（beats per minute, BPM）。（H2）腎上腺素會增加此物種背血管的BPM。繪製你**預期**結果的單一曲線圖在圖7，顯示出**單次**投與A試劑、B試劑和W（PBS 磷酸鹽緩衝洗液）後的**所產生的**效應。務必確認本實驗中不同時間中背血管bpm的變化。

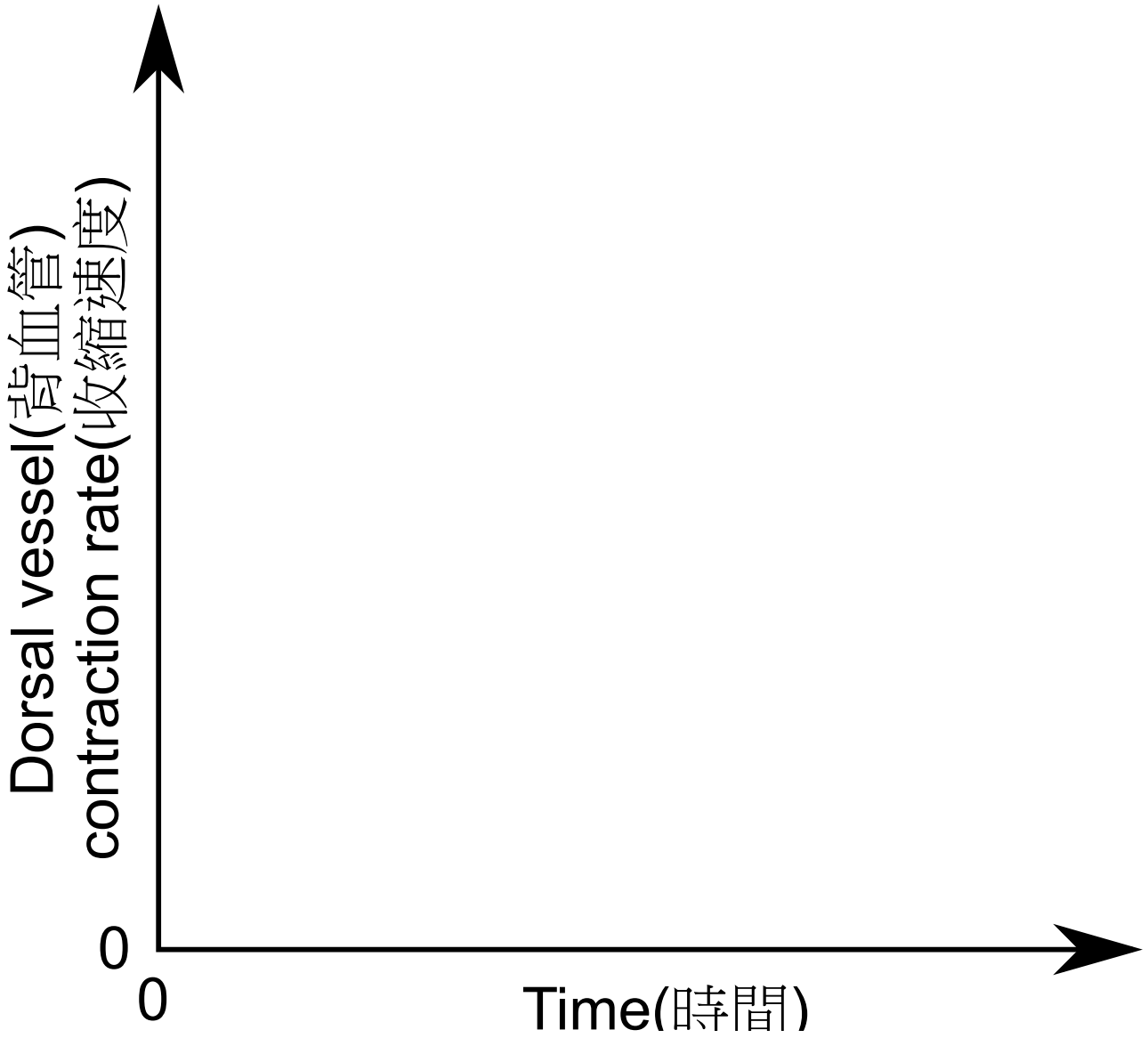

Figure 7. Line graph of expected results for addition of Acetycholine (A) and Adrenaline (B) and PBS wash (W) to the C.vicina dorsal vessel
圖7.將乙醯膽鹼（A）和腎上腺素（B）和PBS洗液（W）添加到C.vicina背血管預期結果的曲線圖

(7 分)

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.
設計並執行一個實驗來測試H1和H2假說，以辨識昆蟲激素八酚胺（C）如何作用於在任務2b中被分離、在任務2c中被確認的背血管。
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.
下面的區塊表示你在單次幼蟲準備中進行理想實驗的持續時間。將該塊分成幾段，來表示您從任務2c的一次幼蟲製備中使用各溶液（A、B和C）和PBS洗液（W）的次序。將正確的字母代碼寫入各區中，前兩個已幫你完成。
3. When designing your experiment you need to consider and effectively represent, washing of the tissue and repetitions.
你的實驗設計要能清楚呈現八酚胺對背血管之作用，清洗掉八酚胺對背血管之作用及呈現實驗的結果是否具有重複性。

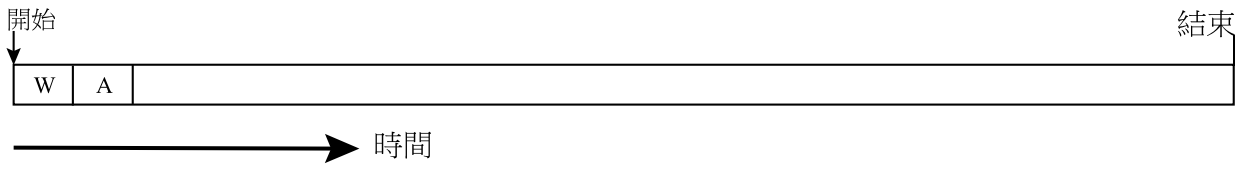

圖8. 實驗設計 。請設計如何添加磷酸鹽緩衝液(PBS)（W）和A ，B ，C三種不同試劑來證實試劑C的作用

(12 分)

任務**2e**

1. Cover your preparation with fresh PBS (W) and record the baseline beats per minute (bpm) in Table 2
   用新鮮的PBS淹蓋你預備好的材料，在表2中記錄靜止狀態下每分鐘的心跳次數（bpm）以為基礎值。
2. Three eppendorfs marked A, B, and C contain stocks of:
   A = 2 x 10-2 M Acetylcholine
   B = 2 x 10-2 M Adrenaline
   C = 2 x 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 x 10-3M in 1 mL in Eppendorf tubes. For each agent record volumes for dilutions in Table 2 in μL.

   標有A、B和C的三Eppendorf小管中含有下列藥物之高濃度原液：
   A = 2×10^{-2} M乙醯膽鹼
   B = 2×10^{-2} M腎上腺素
   C = 2×10^{-2} M八酚胺

   一個醫用藥水瓶中含有100ml PBS
   使用前，在1 mL Eppendorf小管中以PBS稀釋在A、B和C中的溶液至5×10^{-3} M的實際作用濃度。對每種藥物在表2中記錄用以稀釋的體積（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.
   評估你的理想實驗設計（圖8）及可做完的時間，測試A、B、C和W對解剖所得之背血管的影響。將原始數據記錄在所提供的紙上，並在表2中填入背血管在靜止狀態下及在不同藥物A，B和C作用下的平均bpm。
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
   根據你的數據，在表中選擇 bpm的變化，例如增加、在 10%範圍內無變化、或減少
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.
   根據你的數據，在第4行的相關列中分別以“1”或“0”來指出每種藥物的受體“是”或“否”具有活性。

Table 2. BPM of resting tissue, effect of agents A, B and C and identification of agents.
(表二、靜止狀態下，試劑ABC處理後每分鐘之心跳數及各試劑之確認)

溶液	W	A	B	C
Dilution volumes **稀釋體積 (µL)**	Not required 不需要	Stock 高濃度原液所需體積 PBS添加磷酸鹽緩衝液之體積	Stock 高濃度原液所需體積 PBS添加磷酸鹽緩衝液之體積	Stock 高濃度原液所需體積 PBS添加磷酸鹽緩衝液之體積
Record your raw data counts here 在此紀錄你所得到的原始數據 **Average bpm**平均每分鐘之心跳數	Average = 平均=	Average = 平均=	Average = 平均=	Average = 平均=
Select one of the following responses. 下列變化選擇其一	增加 在10%內無變化 減少	增加 在10%內無變化 減少	增加 在10%內無變化 減少	增加 在10%內無變化 減少
藥物受體活性 **1** = 有活性 **0** = 沒有活性				

(12 分)

任務 **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)
   在提供的圖紙上，紀錄你的數據並繪製在不同時間中所得到的結果。記住本圖表應可顯示出你每次的實驗結果。
   （12分）
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.
   H1實驗是指乙醯膽鹼之投予會降低*C. vicina*背血管之跳動速度， H2假設腎上腺素之投予會促進背血管之跳動速度，和哺乳動物的心臟一樣。
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:
   你必須根據實驗所得的數據，接受或拒絕H1及H2之假設。
   在下面圈選你的決定：

H1 ACCEPT 接受 REJECT拒絕

H2 ACCEPT 接受 REJECT拒絕

考試結束