18th INTERNATIONAL BIOLOGY OLYMPIAD
第十八屆國際生物奧林匹亞競賽
JULY 15 - 22, 2007

PRACTICAL EXAMINATION 3　實驗題 3

Cell Biology/Biochemistry　細胞學／生物化學

TASK A.	**Thiocyanate analysis in cauliflower** 分析花椰菜中硫氰鹽含量	**29 marks** **29** 分
TASK B.	**Determination of the amount of cauliflower needed to be consumed to cause toxicity** 計算花椰菜中硫氰鹽的毒性	**5 marks**　**5** 分
TASK C.	**Regulation of gene expression** 基因表現調控	**10 marks** **10** 分

Time allowed: 90 minutes
總時間為 **90** 分鐘

WRITE ALL ANSWERS IN THIS EXAM BOOKLET
將答案書寫於本試卷上

WRITE YOUR 4-DIGIT STUDENT CODE IN THE BOX BELOW AND ON THE TOP OF EACH PAGE OF THIS BOOKLET
將四位數的學生代碼填於下面的空格中，並於每頁的上方重複填寫

STUDENT CODE	

Introduction

The cabbage family contains a class of compounds known as glucosinolates. Some glucosinolates such as glucoraphanin have desired medicinal properties helping to prevent cancers while others such as glucosinalbin have toxic metabolites.
十字花科植物含有一種稱為 glucosinolate 的化合物。有些 glucosinolate 的化合物，例如 glucoraphanin 具有預防癌症發生的功能。但是有些 glucosinolate 的化合物，例如 glucosinalbin ，則會在代謝過程中產生毒性物質。

One of the products of the toxic glucosinolates is the thiocyanate ion (SCN^-). SCN^- interferes with iodine metabolism resulting in thyroid hormone deficiency. Eating plants of the crucifer family such as cauliflower will result in the production of a limited amount of thiocyanate ion from glucosinolates such as glucosinalbin.
具有毒性的 glucosinolate 分子主要是肇因於 硫氰根離子 (SCN^-)。硫氰鹽類會干擾碘離子的代謝，進而造成甲狀腺素的不足。攝食十字花科植物，例如花椰菜的 glucosinalbin（來自 glucosinolates），會導致少量的硫氰鹽類的生成。

The glucosinolate glucoraphanin is metabolized to sulforaphane. Sulforaphane is an inducer of phase 2 proteins. One consequence of phase 2 protein induction is an increased ability of cells to scavenge free radicals and other oxidants. A consequence of decreased oxidant levels is a lower probability of activation of pathways that lead to inflammation. One such pathway is through activation of a protein complex such as NFkappaB.
這些 glucosinolate 類的 glucoraphanin 會經由代謝路徑生成 sulforaphane。這些 sulforaphane 分子屬於第二型蛋白的誘發者。這些第二型蛋白具有清除自由基 (free radicals) 與其他氧化劑 (oxidant) 的功能。當氧化劑濃度被降低後，會減緩發炎反應的活化路徑。其中一種路徑便是活化蛋白質複合物，例如：NFkappaB。

TASK A. To determine the amount of thiocyanate ion released from cauliflower using a spectrophotometric assay. This has a value of 27 marks.
利用分光光度計分析法測定自花椰菜中所示釋放出硫氰基的含量（本題 27 分）

OBJECTIVE: To use a spectrophotometer to determine how much thiocyanate ion is released from cauliflower. This assay is based upon the principle that in an acid environment thiocyanate reacts with Fe^{3+} to form a stable Fe^{2+}-SCN red-coloured complex with a maximum absorption at 447 nm.

目的：利用分光光度計法測定花椰菜所釋出的硫氰鹽類含量。此方法是利用在酸性的環境下，硫氰鹽類會與三價鐵離子 (Fe^{3+}) 反應，形成具有穩定的硫氰鐵分子 (Fe^{2+}-SCN)之紅色複合物，此硫氰鐵分子在 447 nm 波長下具有最大的吸光值。

Materials
材料

- Eppendorf pipettor: one 20-200 microlitre capacity set to 100 microlitres.
  微量滴管：將 20-200 ul 的微量滴管調整到 100 ul

- Eppendorf pipette tips.
  微量滴管吸頭

- Spectrophotometer cuvettes containing 900 microlitres of ferric nitrate reagent – as noted above, this reagent is in a strong acid.
  分光光度管內含 900 uL 硝酸鐵溶液。說明如上，此溶液為強酸。

  CAUTION: The ferric nitrate reagent solution you will be using is dissolved in 1.0 M nitric acid. Wear gloves and use goggles to protect your eyes before starting the experiment.
  注意:此硝酸鐵溶液是用 1.0M 硝酸所製備。在實驗開始前必須配帶保護眼鏡並穿戴手套。

- Thiocyanate standards in tubes at the following concentrations: 0 micromoles/mL (this is your blank), 0.1 micromoles/mL, 0.5 micromoles/mL, 1.0 micromoles/mL, 2.0 micromoles/mL and 4.0 micromoles/mL.
  在試管中的硫氰鹽標準液的濃度分別為 0 uM/mL (本溶液為空白對照組)，0.1 uM/mL, 0.5 uM/mL, 1.0 uM/mL, 2.0 uM/mL 與 4.0 uM/mL

- One tube of filtered cauliflower homogenate. 1.0 g of cauliflower was homogenized and the homogenate was diluted to a total volume of 4.0 mL water. This is your unknown and you will be required to determine how many micromoles of thiocyanate are present in one millilitre of this homogenate.
  試管中裝有花椰菜均質液。將 1.0 g 的花椰菜均質化後，最後將均質液的總體積用水調整至 4.0 mL，此即為未知物（為本次實驗所要測定的溶液）。最後要測定的是 1 mL 溶液中含有多少 mM 的硫氰鹽。

- A marker pen to label the cuvettes.
  以馬克筆在分光光度管註記。

- Gloves and protective glasses
  戴上手套與保護鏡。

- On your bench is a spectrophotometer set to an absorbance of 447 nm.
  請注意你桌上的分光光度計是否是設定在 447 nm。

NOTE: Before beginning this task, be sure that you have all the materials listed above. If you do not, notify a lab assistant by raising your hand.
附註：在本問題開始前請注意是否你的材料與儀器是否完備，如果沒有，請舉手向監考人員提出需求。

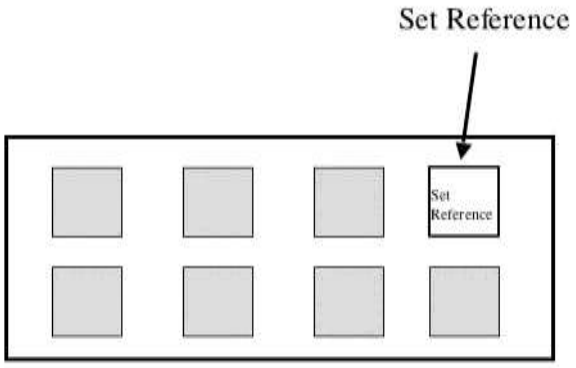
Procedure
步驟

1. Put on the gloves and the protective glasses.
   戴上手套與保護鏡

2. To each of the cuvettes containing the ferric nitrate reagent add 100 microliters of each of the thiocyanate standards. The standards are: 0, 0.5, 1.0, 2.0 and 4.0 micromoles thiocyanate/mL. A coloured reaction should become visible except for the 0 micromole thiocyanate standard which serves as your blank. Be sure to label the cuvettes on the frosted surface.
   分別將濃度為 0, 0.5, 1.0, 2.0 與 4.0 M/mL 的硫氰鹽標準液各取 100ul，分別加入分光光度管中。此時，除了濃度為 0 的標準液外，其他會有呈色反應在分光光度管中進行。濃度為 0 的標準液為本實驗的空白對照組。請注意是否有在分光光度管磨砂面上做上註記。

3. To each of the remaining 3 cuvettes add 100 microlitres of the cauliflower homogenate.
   在剩下的 3 個分光光度管中分別加入各 100 uL 的花椰菜均質液。

4. Carefully carry the cuvettes to the spectrophotometer which has been set to absorb at 447 nm. Open the lid to the light path in the spectrophotometer and insert the 0 micromole thiocyanate/mL standard (i.e., blank) cuvette. The arrow indicates the light path. Ensure that the walls of the cuvellets through which the light passes is transparent. Close the lid and push the "set reference" button on the top right hand of the panel on the spectrophotometer – see the diagram below.
   Do not touch any of the other buttons!
4. 小心地移動分光光度管到分光光度計中，分光光度計的吸收值應該設在 447nm。打開蓋子，插入 0 uM/mL 標準液的分光光度管（本管為空白對照組）。分光光度計裡的箭號，註記的是光束的路徑。請注意光束通過的分光光度管應該是透明的。關上蓋子，按下位於分光光度計操作面板右上方 "set reference" 按鍵，如下圖所示的位置。
   不可觸碰其他的按鈕。



5. Insert each of the standards and record the reading. Then insert each of the cuvettes containing the unknown and record the spectrophotometer reading. Leave the cuvettes at the spectrophotometer and the laboratory assistants will take care of them.

分別依序插入其他的標準液的分光光度管，並分別依序記錄下讀數。此時插入未知濃度的花椰菜均質液之分光光度管，並分別記錄讀數。當所有的分光光度管都分別記錄完成後，將分光光度管留置於該處，監考人員會收拾善後。

Spectrophotometer reading (absorbance) for each standard: This has a value of 10 marks.
標準液的分光光度計讀數（吸光值）這個部分有 10 分。

0.5 micromole/mL thiocyanate: _____
0.5 mM/mL 硫氰鹽溶液

1.0 micromole/mL thiocyanate: _____
1.0 mM/mL 硫氰鹽溶液

2.0 micromole/mL thiocyanate: _____
2.0 mM/mL 硫氰鹽溶液

4.0 micromole/mL thiocyanate: _____
4.0 mM/mL 硫氰鹽溶液

Spectrophotometer reading (absorbance) for the unknown: This has a value of 3 marks.
分別記錄三個未知濃度的花椰菜均質液之吸光值，此題 3 分。

1._____ 2,_____ 3,_____

6. Plot, on the graph paper (on the next page), the absorbance measurements for your thiocyanate standards against the concentration (micromoles/mL) of the standards. **This has a value of 6 marks.**
在下一頁作圖，橫軸為硫氰鹽離子濃度，縱軸為分光光度計讀數（吸光值）。**此題 6 分。**

7. Take the average absorbance of your cauliflower homogenate and determine the thiocyanate ion concentration using the previously plotted graph. **This has value of 5 marks.**
利用前一題所畫出來的圖，將所有的花椰菜均質液的分光光度計讀數（吸光值）平均，並計算花椰菜均質液中硫氰鹽的濃度。**此題 5 分**。

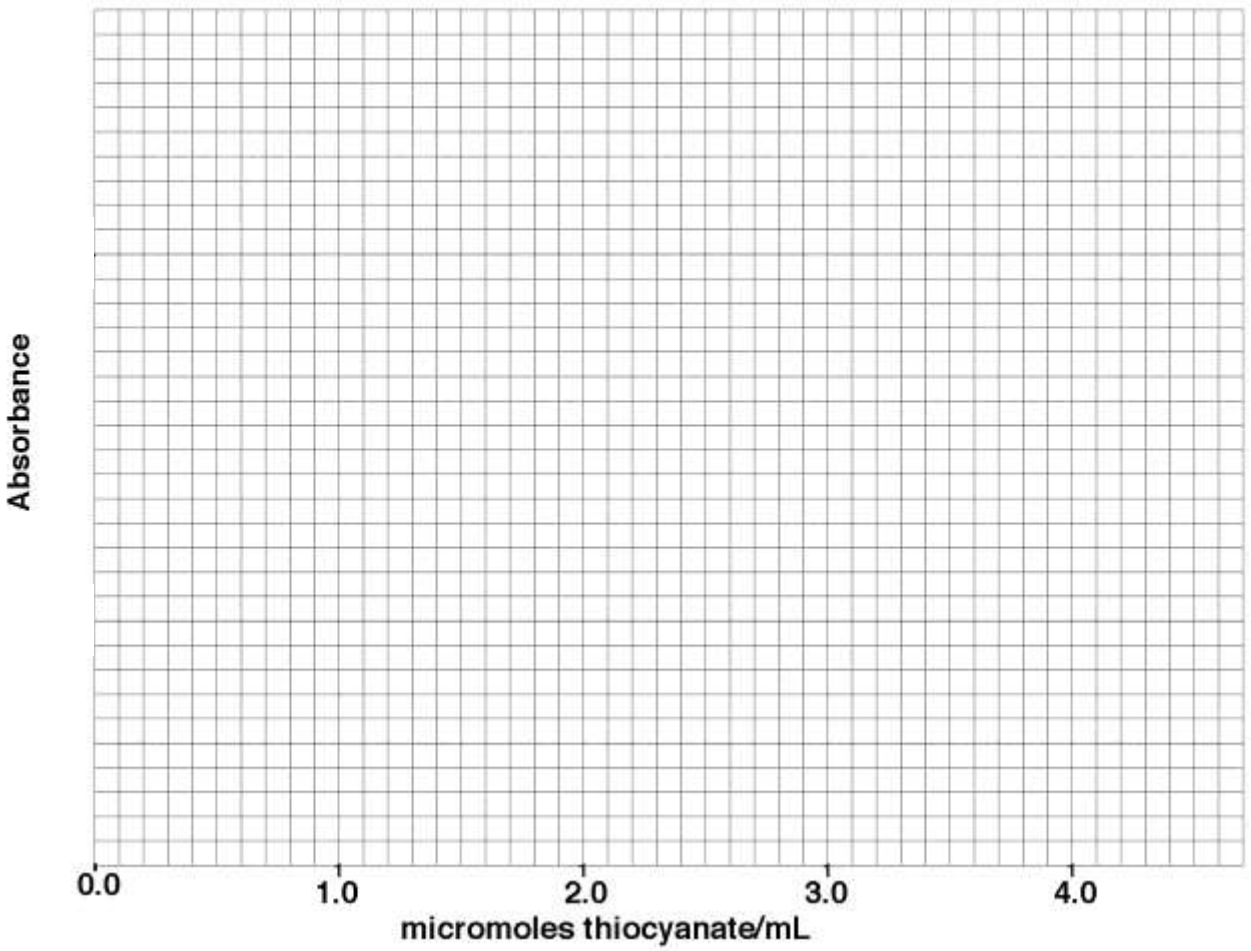
ANSWER: _____

8. What is the concentration of thiocyanate in cauliflower? Be sure to state the units (3 marks)
花椰菜均質液中硫氰鹽的濃度為何？（請標出單位；3 分）

ANSWER: _____

9. What is the standard deviation of the absorbance of the unknown? **This has value of 2 marks.**
花椰菜均質液中硫氰鹽的濃度的標準差為何，**此題 2 分**。

ANSWER: _____


Absorbance
0.0
1.0
2.0
3.0
4.0
micromoles thiocyanate/mL

TASK B. To determine the amount of cauliflower needed to be consumed for it to cause toxic effects because of the presence of thiocyanate (5 marks)

測定花椰菜中硫氰鹽的致死劑量 **(LD_{50})**（**5** 分）

Introduction

The LD_{50} is a toxicology term that describes the dose (i.e. millimoles of toxin/kg animal) of a compound that will kill 50% of the animals tested. In the rat, the LD_{50} of sodium thiocyanate consumed is reported to be 9 millimoles/kg. Using the data of the experiment you have just performed, calculate how much cauliflower a rat that weighs 500 g would have to eat in a short time to reach the LD_{50} of thiocyanate.
LD_{50} 為毒物學中用來計算化合物能殺死 50% 實驗動物的劑量 (mM/kg)，可稱為 50% 致死劑量。在大鼠的實驗報告指出，硫氰鈉的 LD_{50} 為 9 mM/kg。利用問題 A 的實驗結果，請推算體重 500g 的大鼠在短時間內要攝食多少的花椰菜才能達到硫氰鹽的 LD_{50}。

Procedure

Circle the letter of the range that best fits your calculated value. Show your calculations on this page. Continue on the back of this page if necessary.
請圈出最適當的答案。並於試卷中寫出你的計算式。如不敷使用，可以使用本頁背面。

(a) 1 gm to 5 gm

(b) 50 gm to 250 gm

(c) 500 gm to 1 kg

(d) 1.5 kg to 14 kg

(e) 15 kg to 25 kg

TASK C. To interpret the regulation of gene expression. (12 marks)
說明基因表達的調控（12 分）

Introduction
說明

The glucosinolate glucoraphanin is metabolized to sulforaphane. Sulforaphane is an inducer of phase 2 proteins. One consequence of phase 2 protein induction is an increased ability of cells to scavenge free radicals and other oxidants. A consequence of decreased oxidant levels is a lower probability of activation of pathways that lead to inflammation. One such pathway is through activation of a protein complex such as NFkappaB.
這些 glucosinolate 類的 glucoraphanin 會經由代謝路徑生成 sulforaphane。這些 sulforaphane 分子屬於第二型蛋白的誘發者。這些第二型蛋白為具有清除自由基 (free radicals) 與其他氧化劑 (oxidant) 的功能。當氧化劑濃度被降低後，會減緩發炎反應的活化路徑。其中一種路徑便是活化蛋白質複合物，例如：NFkappaB。

NFkappaB is a transcription factor complex comprised of two proteins (p50 and p65) bound to a third protein known as IkappaB that is normally present in the cytoplasm. Activation of NFkappaB involves the degradation of IkappaB resulting in theNFKappaB p50/p65 heterodimer translocating to the nucleus where it binds to specific promoter elements increasing the transcription of pro-inflammatory genes such as inducible nitric oxide synthase (iNOS). One indicator of activation of NFkappaB is that the ratio of the p65 to IkappaB protein increases.
NFkappaB 為轉錄因子複合體的一種，本身能與兩種蛋白質 (p50 與 p65) 結合。當結合兩種蛋白質後能與存在細胞質中的第三種蛋白質 IkappaB 結合。一般狀況下，NFkappaB 能與 p50 蛋白與 p65 結合，再與 IkappaB 結合。活化的 NFkappaB 會參與 IkappaB 的降解，結果會導致 NFkappaB p50/p65 蛋白質複合體轉位到細胞核。在細胞核中，NFkappaB p50/p65 蛋白質複合體會與啟動子單元結合，進而增加前發炎反應基因 (pro-inflammatory gene)，（如誘導型一氧化氮合成酶 (iNOS)）的轉錄。因此，p65 與 IkappaB 的比值可以做為 NFkappaB 的活化指標。

One of the consequences of increased iNOS activity is increased production of the nitric oxide free radical ($NO^{\cdot}$). Nitric oxide reacts with the superoxide anion ($O2^{\cdot-}$) to form peroxynitrous acid. Peroxynitrous acid is a very strong oxidant.
當 iNOS 的活性增加，一氧化氮的自由基 ($NO^{\cdot}$) 的產量也隨著增加。一氧化氮與超氧分子 ($O_2^{\cdot-}$) 作用後會形成過氧硝酸 (peroxynitrous acid)，而過氧硝酸便是一種很強的氧化劑。

Increased oxidant levels often results in activation of NFkappaB while lowering oxidant levels often results in decreased activation of NFkappaB and, hence, lowered levels of expression of pro-inflammatory genes.
NFkappaB 活化後，會導致氧化劑濃度上升。然而，當氧化劑濃度降低後，NFkappaB 也會降低活化程度，間接降低前發炎反應基因 (pro-inflammatory gene)表現。

Procedure

步驟

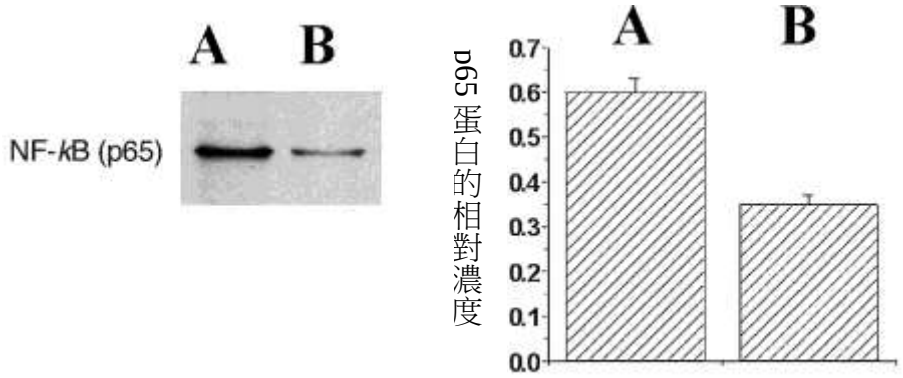
1. Examine the figures provided in each of the following sections.
   仔細檢查以下各問題小節所提供的圖。

2. Using the data presented, identify which data set is derived from animals fed a diet high in glucoraphanin and provide the basis for your answer.
   利用以下的資料，圈選出何者適合作為高單位 glucoraphanin 動物飼料，並以此做為回答問題的基礎。

SECTION A. (5 marks)
Below is a figure that gives data on NFkappaB activation in spontaneously hypertensive stroke-prone (SHRsp) male rats that were fed one of two diets: a control diet or an experimental diet containing glucoraphanin. In the experimental diet, the animals consumed 10 micromoles glucoraphanin/kg body weight.
下圖為 NFkappaB 活化程度，實驗來自雄性原發性高血壓中風大鼠。一組為對照組飲食，另一組為實驗組飲食（添加 glucoraphanin）。在本實驗中，glucoraphanin 添加量為 10ug/kg。

After several months on these diets, the animals were euthanized, nuclei from the kidneys were isolated and prepared for SDS polyacrylamide electrophoresis. Following separation of the proteins on the gel, the proteins were transferred to nitrocellulose membrane and probed with an antibody that recognized the NFkappaB p65 protein.
經過數月的 glucoraphanin 添加飲食的飼養，實驗動物經安樂死處理。腎臟細胞的細胞核被分離出來，並經由 SDS-PAGE 電泳分離。蛋白質會在膠體中被分開，這些蛋白質會被轉印到硝化纖維紙上，並經由抗體辨識 NFkappaB p65 蛋白。

A representative Western blot is shown below (on the left) and next to it is a graph that depicts the quantification of blots from 5 different animals per diet group.
左圖為西方墨點法的實驗結果，右圖為分別量化五隻實驗動物經由上述西方墨點法的實驗結果。



Answer the following questions:
回答下列問題

1. Which group of animals **(A or B)**, were fed the glucoraphanin-containing diet? This has a value of 1 mark.
1. 上述 A 與 B 兩組實驗動物，何者的飲食中有添加 glucoraphanin？**本題 1 分**

ANSWER: _____

2. Which of the following statements gives the best explanation for your answer? Circle the letter of that statement. This has a value of 4 mark.
請圈出下列何者為最適合本實驗的說明。**本題 4 分**

(a) Less oxidative stress results in less NFkappaB activation and hence less p65 in the nuclei.
較低的氧化壓力會導致 NFkappaB 的活化降低，因此降低 p65 在細胞核的表現。

(b) Less oxidative stress results in less NFkappaB activation and hence more p65 in the nuclei.
較低的氧化壓力會導致 NFkappaB 的活化降低，因此升高 p65 在細胞核的表現。

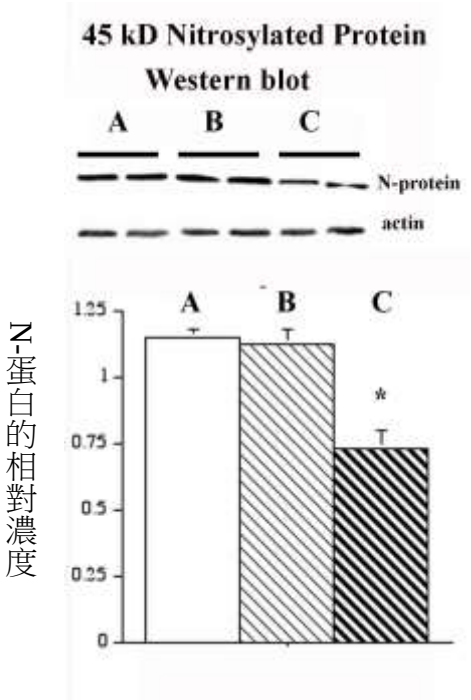
(c) More oxidative stress results in less NFkappaB activation and hence less p65 in the nuclei.
較高的氧化壓力會導致 NFkappaB 的活化降低，因此降低 p65 在細胞核的表現。

(d) More oxidative stress results in less NFkappaB activation and hence more p65 in the nuclei.
較高的氧化壓力會導致 NFkappaB 的活化降低，因此升高 p65 在細胞核的表現。

(e) More oxidative stress results in more NFkappaB activation and hence more p65 in the nuclei.
較高的氧化壓力會導致 NFkappaB 的活化增加，因此升高 p65 在細胞核的表現。

SECTION B. (5 marks)
Below is a figure that gives data on a 45 kD nitrosylated protein (N-protein) in the kidneys of male SHRsp rats that were put on one of three different diets: a diet containing glucoraphanin and two different control diets.

下圖為一個 45kD 亞硝醯化蛋白 (N-蛋白) 的實驗結果。實驗來自雄性原發性高血壓中風大鼠。實驗動物被設計成三種不同的飲食，一組添加 glucoraphanin，其他兩組為不同的對照組飲食。



The top part of the figure is a representative Western blot while the bottom part of the figure is the quantification of Western blots from 5 different animals per diet group.
上圖為西方墨點法的實驗結果，下圖為分別量化五隻實驗動物經由上述西方墨點法的實驗結果。

Answer the following questions:
回答下列問題
1. Which of the groups, A, B or C, represent the animals fed a diet containing glucoraphanin? This has a value of 1 mark.
上述 A, B, C 三組實驗動物，何者的飲食中有添加 glucoraphanin？**本題 1 分。**

ANSWER: _____

2. Circle the letter of the statement below that best explains your answer. Glucoraphanin treatment results in: (4 marks).

在經由 Glucoraphanin 處理之後，請圈出下列何者最適合本實驗所得的結果。**本題 4 分。**

(a) More oxidative stress results in more NFkappaB activation that results in more iNOS expression and more peroxynitrous acid formation and thus more nitrosylation of proteins.
較高的氧化壓力會導致 NFkappaB 的活化增加，因此導致較高的 iNOS 表現與較高的過氧化硝酸生成，於是蛋白質的亞硝醯化增加。

(b) More oxidative stress results in more NFkappaB activation that results in more iNOS expression and more peroxynitrous acid formation but less nitrosylation of proteins.
較高的氧化壓力會導致 NFkappaB 的活化增加，因此導致較高的 iNOS 表現與較高的過氧化硝酸生成，於是蛋白質的亞硝醯化降低。

(c) Less oxidative stress results in more NFkappaB activation that results in more iNOS expression but less peroxynitrous acid formation and thus less nitrosylation of proteins.
較低的氧化壓力會導致 NFkappaB 的活化增加，因此導致較高的 iNOS 表現與降低的過氧化硝酸生成，於是蛋白質的亞硝醯化降低。

(d) More oxidative stress results in less NFkappaB activation but results in less iNOS expression and less peroxynitrous acid formation and thus less nitrosylation of proteins.
較高的氧化壓力會導致 NFkappaB 的活化降低，因此導致較低的 iNOS 表現與降低的過氧化硝酸生成，於是蛋白質的亞硝醯化降低。

(e) Less oxidative stress results in less NFkappaB activation that results in less iNOS expression and less peroxynitrous acid formation and thus less nitrosylation of proteins.
較低的氧化壓力會導致 NFkappaB 的活化降低，因此導致較低的 iNOS 表現與降低的過氧化硝酸生成，於是蛋白質的亞硝醯化降低。

- THE END –
結束

HAVE YOU WRITTEN YOUR STUDENT CODE ON THE FIRST PAGE OF THIS EXAM BOOKLET AND ON THE TOP OF THE OTHER PAGES?
請確認是否有將四位數的學生代碼填於第一頁的空格中, 並於每頁的上方填寫