

1, 2, 3: Single-choice questions.

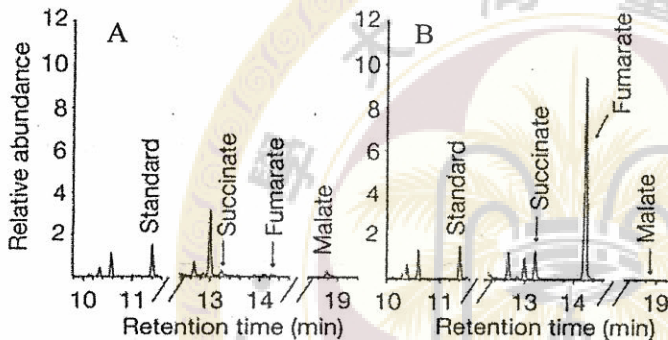
1. Telomerase elongates

- (A) the template RNA strand at the 3' end (B) the template DNA strand at the 5' end
(C) the template DNA strand at the 3' end (D) the template RNA strand at the 5' end. (2%)

2. Which of the following molecules is an intermediate in the catabolism of adenine and guanine?

- (A) hypoxanthine (B) xanthine (C) uric acid (D) all are right (E) none of them. (2%)

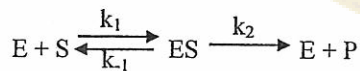
3-4. Fumarate hydratase (FH) is an enzyme of the tricarboxylic acid cycle (TCA cycle) that catalyses the hydration of fumarate into malate. To study how important of FH to renal-cell cancer, Mu-Hsin generated an immortalized mouse kidney cell line which contains homozygous *Fhl*-deleted (knockout) alleles. A GC-MS (gas chromatography-mass spectrometry) analysis was performed to confirm the cell line, and the representative chromatogram of a GC-MS analysis was shown as follows. Annotated metabolites are indicated by the arrows.



3. According to the figure above, which one is the *Fhl*-deleted cell line? (A) or (B). (2%)

4. If Succinate dehydrogenase-coding gene is deleted, what will happen? Please use the figure above as a reference to draw a predicted figure. (4%)

5. Please provide a step-by-step derivation of the Michaelis-Menton equation for the kinetic scheme shown below: (4%)



6. Tight binding between enzyme and substrate is considered bad for catalysis, why? (3%)

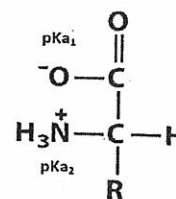
7. You are trying to clone a human gene by using a brain cDNA library as the PCR template. Although the primers for PCR are correctly designed, you failed to amplify the target gene. Given this negative result, what would you try next? (3%)

8. Please draw the hydrogen bond between a dG-dC pair in a strand of DNA (assume it follows the classical Watson-Crick pairing rule). Use this to explain at which position of the cytosine you can put a small additive such as a Cy5 fluorophore to preserve the integrity of the base pairing. (10%)

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9. Give the approximate molecular masses of an immunoglobulin G molecule analyzed by (a) gel filtration chromatography, (b) SDS-PAGE, and (c) SDS-PAGE in the presence of 2-mercaptoethanol. (4%)
10. Which peptide has greater absorbance at 280 nm? (2%)
A. Ser-Val-Trp-Asp-Phe-Gly-Tyr-Trp-Ala
B. Gln-Leu-Glu-Phe-Thr-Leu-Asp-Gly-Tyr
11. You have isolated a protein from the bacterium *E. coli* and seek to confirm its identity by trypsin digestion and mass spectrometry. Determination of the masses of several peptide fragments has enabled you to deduce the identity of the protein. However, there is a discrepancy with one of the peptide fragments, which you believe should have the sequence MLNSFK and an $(M+H)^+$ value of 739.38. In your experiments, you repeated obtain an $(M+H)^+$ value of 767.38. What is the cause of this discrepancy and what does it tell you about the region of the protein from which this peptide is derived? (4%)
12. How dietary cholesterol is absorbed and transported to the liver? (5%)
13. Please explain the functional roles of adipose tissue in terms of lipid metabolism. (5%)
14. Please describe the critical structures of mRNA and tRNA and their functions in prokaryotic translation (6%)?
15. Please describe 4 regulatory mechanisms involved in eukaryotic transcription (4%)?
16. Please describe how glucagon modulates glycogen metabolism, glycolysis and gluconeogenesis? (6%)
17. How does carbohydrate metabolism play important roles in fatty acid and amino acid biosynthesis? (4%)
18. Given the following facts about isoelectric point of an amino acid: 1) the amino acid has two dischargable protons (pK_{a1} and pK_{a2}) and 2) an isoelectric point pI is the pH where the amino acid has a zero net charge. Derive the equation (below) that calculates the isoelectric point of the amino acid. (10%)

$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$



19. A persistent inflammatory reaction is a hallmark of chronic and acute pathologies in the central nervous system (CNS) and greatly exacerbates neuronal degeneration. The proinflammatory cytokine tumor necrosis factor α plays a pivotal role in the initiation and progression of inflammatory processes provoking oxidative stress, eicosanoid biosynthesis, and the production of bioactive lipids. We established in neuronal cells that TNF- α exposure dramatically increased magnesium-dependent neutral sphingomyelinase activity thus generating the bioactive lipid mediator ceramide essential for subsequent NADPH oxidase activation and oxidative stress. Since many of the pleiotropic effects of ceramide are attributable to its metabolites, we examined whether ceramide kinase, converting ceramide to ceramide-1-phosphate, is implicated both in NADPH oxidase activation and enhanced eicosanoid production in neuronal cells. In the present study, we

demonstrated that TNF α exposure of human SH-SY5Y neuroblastoma caused a profound increase in ceramide kinase activity. Depleting ceramide kinase activity using either siRNA or pharmacology completely negated NADPH oxidase activation and eicosanoid biosynthesis yet, more importantly, rescued neuronal viability in the presence of TNF- α . These findings provided evidence for a critical function of ceramide-1-phosphate and thus ceramide kinase activity in directly linking sphingolipid metabolism to oxidative stress. This vital role of CerK in CNS inflammation could provide a novel therapeutic approach to intervene with the adverse consequences of a progressive CNS inflammation.

After reading this paragraph, please fill in the blank with appropriate words. (4%)

_____ regulates _____-stimulated redox-enzyme activity and bioactive lipids biosynthesis in neuroblastoma cells

20. Please describe three applications of phosphatases in biotechnology. (6%)
21. Please describe the steps involved in tumor metastasis? (4%)
22. Please design an experiment to clone out metastasis associated genes from cDNA library.(6%)

