**University of Toronto**
**Faculty of Applied Science and Engineering**

**Final Examination, April 14, 2000**

**Fourth year – Program 05**

**BME496S – Cellular Bioengineering**

**Exam type: C**

**Examiner – P.W. Zandstra**

**Instructions: Read the whole exam before attempting any of the questions. Answer only one of questions 3 and 4.**

**Good Luck!**

1. *[20%]* You have isolated a smooth muscle cell preparation that contracts when a drug (ligand) is applied. The muscle is connected to a force transducer that allows you to measure the force of contraction (cell response). The maximum force of contraction that the preparation is capable of is 1 N. A drug concentration of $1 \times 10^{-8}$ M produces a contraction force of 0.75 N.

   a) Determine the $K_D$ of the drug.

   b) You add a ligand that competitively inhibits muscle contraction. When the ligand is added at a concentration of $5 \times 10^{-7}$ M the contraction force is reduced to 0.25 N. Determine the $K_D$ value for the inhibitor.

   c) What concentration of the drug is necessary to achieve the original contraction force of 0.75 N in the presence of $5 \times 10^{-7}$ M of the inhibitor?

2. *[20%]* The 72 h dose - growth responses of two ligands, EGF and TGF, on the same cell population under the same conditions are shown in Figure 2a. Both ligands signal through the same receptor.

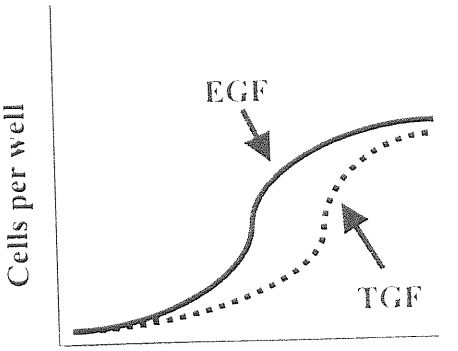

Figure 2a

Now consider the following idealized and simplified model for MAPK pathway activation by growth factor (gf.) receptor (C*) signaling. Activation of receptor signaling (which then reversibly generates active MAPK (S*) from inactive MAPK (S)) can be assumed to occur essentially immediately upon ligand binding, followed by a desensitization to an inactive complex which can then dissociate the ligand.

$$L + R \underset{k_r}{\overset{k_f}{\Leftrightarrow}} C^* \xrightarrow{k_{desen}} C_{inact} \xrightarrow{k_r} L + R$$

$$S \underset{k_{-1}}{\overset{k_1 C^*}{\Leftrightarrow}} S^*$$

a) Assuming that the cell mitogenic response is related to the level of S*, can you use this model to explain how the hierarchy in mitogenic potency among the two ligand complexes could arise from their corresponding levels of cell-surface receptor complexes? (Note: do not add unnecessary complexity to your solution, e.g., you should assume no gf. depletion, no receptor down regulation, etc.)

b) Predict the order of the ligands in terms of increasing $K_D$.

See Figure 2b. This figure is a set of data showing cell proliferation (plotted as average growth rate) during incubation with the same two growth factors maintained at a constant concentration.

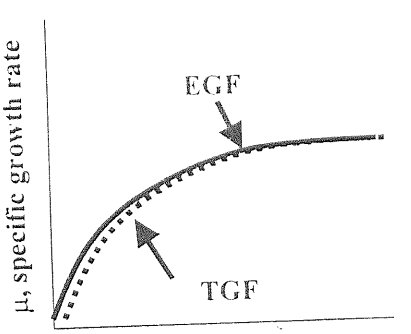

Figure 2b

c) Can this set of data be reconciled with your explanation from part a)? Explain how or why not.

**NOTE: PLEASE CHOOSE EITHER QUESTION 3 OR 4.**
**DO NOT DO BOTH**

3. *[20%]* The cell migration flux expression developed in class consisted of a "dispersion term" and a "biased migration" term.

i) Develop the equation for cell flux ($J_c$) along a gradient axis ($x$) in terms of the dispersion term, the chemotatic term and the chemokinetic term.

ii) Define the meaning of the $\frac{\partial L}{\partial x}$, $N$, $\frac{1}{2}\frac{du}{dL}$ terms in the equation.

iii) Draw a sketch of cell population migration in an attractive gradient. Label, on your diagram, all the terms you have developed in your equation for cell flux.

iv) An experiment is done under conditions where the chemotactic term is zero, yet the cells are found to accumulate at the higher concentration end of the gradient. Briefly explain why this may be so.

**OR**

4. *[20%]* It has been hypothesized that transferrin, and not oxygen, is the limiting nutrient for encapsulated islet cells. Representative transport and reaction data for oxygen and transferrin are as follows:

| Component | Q (mol/cm$^3$-s) | $D_{tissue}$ | Bulk Conc. (mol/cm$^3$) |
|---|---|---|---|
| Oxygen | $3 \times 10^{-8}$ | $2 \times 10^{-5}$ | $1.6 \times 10^{-7}$ |
| Transferrin | $1 \times 10^{-12}$ | $3 \times 10^{-7}$ | $5 \times 10^{-9}$ |

i) Estimate the concentration of each component in the center of a 150 μm diameter spherical islet relative to the concentration at the surface. You may assume no mass transfer resistance is present in the bulk.

ii) Based on your results, which component do you think is the limiting nutrient for encapsulated islet cells.

5. *[20%]* The introduction of genes to cells to permanently change their genotype has a very large clinical potential. Despite significant efforts, success in this technology has been limited. A major reason for this may be our insufficient understanding of the barriers to gene delivery.

i) Using a diagram, outline what you predict are the 3 major barriers (of the ~7 that were discussed in class) to gene delivery.

ii) Develop realistic rate equations for these barriers.

iii) Describe how you would experimentally determine the rate constants for your 3 major barriers.

6. *[20% total]* Short answer questions.

**You should spend a maximum of 7.5 min. on each of these questions**

*A. [5%]* Cells take up epidermal growth factor (EGF) from the extracellular medium by receptor-mediated endocytosis and horse radish peroxidase (HRP) by fluid-phase endocytosis. An example of the cell uptake of EGF and HRP as a function of the concentration in the medium is shown in Figure 6A.

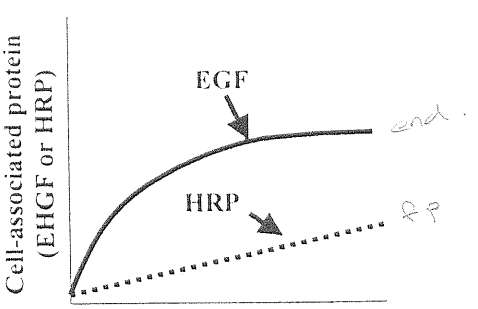


Figure 6A

i) Give a feasible explanation of why the uptake of HRP is linear while the EGF uptake is hyperbolic.

ii) You also notice that the rate of uptake of EGF is much faster than that for HRP. Explain this effect.

B. *[5%]* Outline the experimental protocol you would use to determine $K_{eC}$. How would this protocol be different if you wanted to determine $K_{eR}$.

C. *[5%]* Both biological - and engineering – based approaches are being explored as strategies to overcome organ / tissue / cell rejection. List two biological approaches and one engineering approach. For the engineering approach you have listed, outline two design criteria important for its success.

D. *[5%]* In attempting to understand how mechanical forces might be transduced into biochemical events via cell adhesion receptors, it is proposed that the disassociation rate constant for reversible protein/protein linkages in an intracellular assembly might be increased by imparted stress according to the following expression:

$$k_r = k_{r0} \exp\left\{\frac{\gamma F}{k_B TN}\right\}$$

where $k_{r0}$ is the rate constant under stress-free conditions, F is the stress imparted to the assembly of linkages, $\gamma$ is the linkage interaction distance over which the stress acts, and N is the number of independent linkages present in the assembly.

Using this expression, and a simple kinetic mass action equation for the numbers of bonds ($n_b$) forming between a cell and a substrate, show how bond force is inversely related to $K_D$, the equilibrium dissociation rate constant.