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**BEH.430/2.795//6.561/10.539/HST.544**

## **Homework Set 3**

**Handed out: Friday, Oct. 1, 2004**

**Due: Friday, Oct. 8 by 5pm**

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### Problem 3.1 – Boyden Chamber Assay

Consider the popular Boyden chamber assay for measuring leukocyte chemotactic response to a chemoattractant, in which a quasi-steady-state linear attractant concentration gradient is established for a number of hours across a porous matrix (see motivating paper, Schagat, T. L. et al. "Surfactant protein A differentially regulates peripheral and inflammatory neutrophil chemotaxis." *Am J Physiol Lung Cell Mol Physiol* 284 (2003): L140–L147.)

The matrix is situated between two reservoirs of media at the top and bottom of volumes  $V_t$  and  $V_b$ , respectively. The cells are placed on the top of the matrix to migrate from there ( $x=0$ ) to the bottom ( $x=L$ ). The cell density at the top of the matrix is sufficiently great that it remains approximately constant,  $C_0$ . Upon reaching the bottom of the matrix, the cells quickly fall off onto a filter paper. Thus, the number of cells collected on the filter over any desired time period can be counted. The experiment can be run with an attractant gradient of any magnitude, either from bottom-to-top or top-to-bottom, or with no gradient at all (i.e., with uniform attractant concentration of any level across the matrix).

- a) Set up the appropriate leukocyte density conservation equation and boundary/initial conditions to model this assay presuming that the experiment will be conducted largely during the time-period during which the attractant concentration gradient is roughly steady. Also you can assume that the time-scale of the experiment is sufficiently short that there is no leukocyte death, and that the leukocyte density is sufficiently low that there attractant update is negligible.
- b) Assuming that the leukocyte random motility coefficient,  $D_c$ , and chemotaxis coefficient,  $\chi$ , are constants (independent of attractant concentration), derive an expression for the number of cells collected on the bottom filter once they reach a steady-state distribution within the matrix, as a function of the magnitude of the attractant concentration gradient. Show how this could be used to determine a value for  $\chi$ . Also, in this case try to ascertain a criterion for the matrix thickness,  $L$ , for which this steady-state condition would be valid.

Extra credit: Solve this problem without the steady-state assumption of leukocyte distribution in the matrix; i.e. derive an expression for the number of cells collected on the bottom filter vs. time as a function of the magnitude of the attractant concentration gradient, during this period over which the attractant gradient is roughly steady. Show how this could be used to determine a value for the chemotaxis coefficient (This is a good exercise for solving PDEs.)

- c) It is conceivable that the cells are not actually able to perceive attractant concentration gradients and bias their movement correspondingly, but instead can only alter their random motility in response to the attractant concentration magnitude. In other words, their chemotaxis coefficient might be zero, but their random motility coefficient could vary with attractant concentration. Would this assay be able to distinguish between these two different cell biological mechanisms?

Examine this by recasting the problem with  $\chi = 0$  and  $D_c = D_{co} \cdot (1 + p \cdot a)$  where  $p$  is a coefficient characterizing the change in cell random motility with attractant concentration. Examine how the leukocyte density conservation equation compares with the chemotaxis mechanism and consider that  $p$  might be positive or negative. Explain the associated effects on the leukocyte density distribution across the attractant gradient in the matrix. (Don't solve, just analyze the physical situation behind the parameters.)

### Problem 3.2 – Iterative solution procedure for nonlinear differential equations

A Green's Function approach can be used to develop an iterative solution procedure for nonlinear differential equations. Consider the situation in Deen 2.10, except with a full, non-linear Michaelis-Menten reaction rate for oxygen metabolism (instead of using the 0<sup>th</sup>-order approximation):

$$R = \frac{k_{\max} c}{K_M + c}$$

For the first step, redefine the dependent variable to be  $c' = c - c_0$  in order to render the boundary conditions homogeneous (which is necessary for the Green's Function determination). Then, with this reformulation, rewrite the differential equation as:

$$\mathcal{L}c' = \frac{D}{r^2} \frac{d}{dr} \left( r^2 \frac{dc'}{dr} \right) = f(r) = \frac{k_{\max} [c'(r) + c_0]}{K_M + [c'(r) + c_0]}$$

with corresponding boundary conditions:

$$\begin{aligned} r = r_0, \quad c' &= 0 \\ r = 0, \quad \frac{dc'}{dr} &= 0 \end{aligned}$$

Now, one can take an initial "guess" for the solution  $c'(r)$ , say  $c'_i(r) = 0$ , and treat the right-hand side of the differential equation as a regular heterogeneity to solve for the next iterative guess,  $c'_{i+1}(r)$ , such that

$$\frac{D}{r^2} \frac{d}{dr} \left( r^2 \frac{dc'_2}{dr} \right) = \frac{k_{\max} [c'(r) + c_0]}{K_M + [c'(r) + c_0]} = \frac{k_{\max} c_0}{K_M + c_0}$$

Give this a try. Under what conditions do you believe this procedure—perhaps including further analogous iterations—would provide a useful solution?

### Problem 3.3 – Periodontal infection model with uniformly distributed vasculature

Consider the periodontal infection/inflammation situation discussed in class this past week. We formulated a model on the basis of assuming the focused location of the vasculature at a midpoint in the tissue, so that the cells entered the tissue from the bloodstream while the attractant entered the bloodstream from the tissue at a single spatial location. On the other hand, we noted in passing that this situation could be formulated into a model in an alternative manner – with the vasculature instead assumed to be distributed uniformly throughout the tissue so that the cells could enter the tissue and the attractants could enter the bloodstreams everywhere. Use your imagination to develop the appropriate model equations with all the attendant simplifying assumptions we employed in class for that original formulation. Explore **analysis of the model** to your heart's desire.