Exam instructions.

Put your NAME on all pages.

The exam looks long but there is a lot of data given to you – everything you will need.

Be as BRIEF as possible in answering questions.

Part 1 are short answers. One word may be sufficient. One equation or one diagram might work also.

Parts 2-4 are more involved.

Please be mindful of time. I have broken down the time allotment for you according to the point distribution. Use this as a guide.

Read the whole scenario carefully.
Please be careful to answer what is asked.
In parts 2-4, I have used “ *** ” to alert you that a question must be answered.
Short answers and diagrams or equations are sufficient here as well.

To paraphrase your parents– it took me a lot longer to write than it will take you to answer. Good luck

-Evan
Part 1. (15 points) Short answer questions. (each one is worth 1.5 points – spend no more than 1 min and 48 seconds on each item in part I.)

a. Oncologists (cancer doctors) often use $^{18}$FDG scans to look for tumors because $^{18}$FDG is a tracer for what biological molecule?

   glucose

b. This tracer is being used to detect an increase in what process? What must we know about the behavior of FDG relative to the molecule that it is tracing?

   metabolism; need to know that FDG uptake and metabolism, if not identical to, is at least proportional to glucose uptake and metabolism (through phosphorylation by glucose-6-phosphatase.)

c. Patients are instructed to fast for 12 hours prior to the PET exam to avoid what? (two possible answers – give one)

   saturation of transporters that take glucose from blood to tissue. (alternate answer, enzyme inside cell could, theoretically, be saturated as well)

d. Some tumors show high degrees of angiogenesis – growth of blood vessels. That is, more vessels per ml of tissue. What imaging approach could we use to detect such a phenomenon?
increase in blood vessels per tissue would increase CBV (cerebral blood volume)

e. What model would we use? (You can use a few words, a diagram, or an equation to answer.)

input-ouput model with dynamic CT or MR and contrast agent.

f. A new gene therapy has been invented to help people, whose brains are low in serotonin, synthesize more serotonin receptors and become happier. We’d like to measure the amount of serotonin receptor (protein) that is transcribed once the patient’s brain cells are transfected with the gene for serotonin receptor. If we only know how to image dopamine receptors with PET, but we are experts at molecular biology (i.e., good at manipulating genes), how could we do this (one sentence)?

create a vector that contains two genes, one for serotonin receptor and one for dopamine receptor. Then figure out a way to transfec brain cells. Image the brain with a tracer that binds to the dopamine receptors and use that as a marker of transcription of the serotonin receptor gene.

g. In what area of the brain would the approach suggested in (f), above, be impractical (i.e., not a good idea) and why?

not a good idea in places like the striatum where there is already a high density of dopamine receptors.

h. If a certain genetic disease leads to growth of brain tissue with capillaries that are extra long and kinked (i.e., bent) without altering overall vascular volume, we might use dynamic MR or CT and look for changes in what parameter?

MTT (mean transit time.)
i. We know that the permeability of the extra long blood vessels is normal. We scan the people’s heads with PET using 15-O-water and then 15-O-butanol – both under high flow conditions (F > 100 ml/min/gm) - expecting to see that $K_1$ maxes out at 100 ml/min/g for uptake of 15-O-water. But to our surprise, uptake of 15-O-water seems to track very well with uptake of 15-O-butanol even at high flows. What’s going on? (Just a few words, please.)

At high flow, $E \sim PS/F$. But if $P$ is otherwise low, it might be overcome by unusually high $S$. The point is that we $PS$ is permeability TIMES surface area.

j. A new user of PET does a PET study. He infuses tracer throughout the 1-hour scan in order to achieve a constant level of tracer in the tissue (this works). Yet, when he looks at the data recorded by the PET scanner, there appears to be more noise in the data at the end of the study as opposed to at the beginning (all time frames are the same). Why?

Even though the infusion (as opposed to bolus injection) achieves a steady level of tracer (in molar quantity), there is no holding back radioactive decay. As radioactive decay progresses, there are less counts per a given time frame. Since PET data follow Poisson statistics, Variance(PET) = Mean(PET) as the Mean (counts) get smaller, the SNR which is merely the ratio of Mean/SD goes with Mean/(Mean)$^{0.5}$ = Mean$^{0.5}$

Thus, as the Mean number of counts declines, the SNR declines and the data become noisier (and appear noisier too.)
(35 points) Part II. Experimental scenario and data analysis.
(spend no more than 40 min. on part II)

Methylphenidate is a drug used for treating ADHD that causes enhanced effect of dopamine by blocking re-uptake of dopamine from the synapse (so, on average, there is more dopamine in the synapse in the presence of the drug than without it). Dr A--- wanted to use PET to construct a dose-response curve for the dopamine response to methylphenidate (MP). (Having such a dose response curve might aid in determining the minimum effective dose for drug.)

Dr A--- scanned subjects 5 different times at 4 different doses of MP and once with NO MP, each time using the tracer, $^{11}$C-raclopride, a tracer that binds to the D2 type dopamine receptor.

The data looked like this (the vertical lines show the data +/- standard deviation based on 8 subjects):

![Figure 1](image)

Experiment:
Subjects received a dose of MP at time (~15) min and then were scanned (starting at t=0) for one hour. Subjects were injected with at trace amount of very high specific activity tracer. Dr. A---- measured arterial concentration of tracer ($^{11}$C-raclopride) during the study, and acquired dynamic PET data over the entire 1 hour scan. He made curves of PET radioactivity vs time for the striatum in each subject,. He used the following receptor model to analyze the data:

![Diagram](image)

Unfortunately, Dr A----’s graduate student forgot to label the y-axis of the data.
(A) ***What is the most likely parameter that is being plotted on the Y-axis?
I was looking for either Binding Potential (=k3/k4 = B’max/KD), or Bmax, or simply k3. Any of those are “parameters” of the model.)

The problem with answering Bmax is that the model specifies only a k3 and a k4 as model parameters.

(B) ***What is the phenomenon being measured?

Competition between dopamine and 11C-raclopride for binding to D2 receptors

***Why does the plot ‘flatten out’?

The number of D2 receptors saturates at high levels of the drug – which presumably cause release of greatest amounts of Dopamine.

(C) ***What limitation is there to our interpretation of the results given the parameter you have chosen? (I.e., why might there be more than one interpretation for the results?)

k3 or Binding Potential are BOTH compound parameters. so with k3, for example, one could not know for sure that the drug had not affected the binding rate constant for raclopride, k_{on}, rather than blocked up available receptor sites.

Similarly with interpretation of apparent lowering of Binding Potential.

(D) Dr. A---- learns that the radio-chemists who made the tracer realize that they miscalculated the specific activity (SA) of the tracer for ALL the experiments in which the subjects got 4 gm MP / kg bodyweight. It turns out that the SA was actually much lower than originally calculated.

***Given this distressing information, what is a possible alternative interpretation for the graph in Figure 1?

If low SA, then HIGH MASS of cold tracer. Thus, unusually high mass of cold raclopride could have blocked receptors instead of dopamine released by MP causing premature saturation.

(E) Dr A---- has lunch with Dr. B----- who is an expert in the systemic metabolism (i.e., in the entire body) of raclopride. Dr. B--- informs him that raclopride can be broken down to rac and ^{11}C-pride in the liver of some subjects.
These byproducts are dumped back into the blood stream. Luckily, neither constituent can cross the blood-brain-barrier. Nevertheless, Dr A--- is worried.

***Which byproduct of $^{11}$C-raclopride worries Dr. A---? Why.

$^{11}$C-pride – it does NOT contribute to the PET signal since I said it didn’t cross the blood brain barrier but it is radioactive and in the blood so it will corrupt the input function we need to solve our model.

***What must Dr A--- do to correct the analysis of his data?

measure the metabolic product $^{11}$C-pride in the blood somehow (GC-Mass Spec ?) and then use the findings to subtract the $^{11}$C-pride-related radioactivity to get an input function that represents just native $^{11}$C-raclopride in the blood (plasma)

(F) When Dr A---- looks at his PET data very closely, he notices that all the model fits to the data look like Figure 2. He is not completely satisfied.

![Figure 2](image)

Radioactivity in Striatum per ml of tissue.

time (min)

He also notes that even in the experiments where the specific activity was lower than expected, his model-fits look like Figure 2. (Circles are data, solid curve is a fit of the model.) Dr. A---- decides to add some terms to his model to try to improve the fit.

***What should he add? ***Please draw the new model.

Nonspecific binding. I said that even when SA is low the curves are too low at the end. So, this is not about binding to the receptor. Nonspecific is “non-displaceable” binding. The model needs another term that allows for greater retention of tracer that does NOT depend on more dopamine receptors.
**(G) Write the new mass balance equation(s) - just the equation(s) for what you added.

\[
\frac{dF(t)}{dt} = K_1 C_p(t) - k_2 F(t) - k_{on} \left[ B_{max} - B(t) - B^{DA}(t) \right] F(t) + k_{off} B(t) - k_5 F + k_6 NS
\]

\[
\frac{dNS(t)}{dt} = k_5 F - k_6 NS
\]

(20 points) Part III. Model output  (spend no more than 24 min. on part III)

(A) Uptake of a diffusible tracer, ‘Tracer Q’ from the blood into bone is modeled with the three-tissue compartment model as follows:

\[\text{Plasma} \rightarrow \text{Free} \rightarrow \text{Bound (specific)} \]

Some uptake and retention of Tracer Q is due to binding to the (limited quantity of) Q-receptors on the surface of chondrocytes (bone cells); some retention is due to sticking of Tracer Q to various other (unlimited) components of the cell surface.

***Please label the four boxes clearly and label all arrows in Figure 3.

(B) Because bone is poorly vascularized, the blood volume fraction of bone is negligible. Figure 4 shows a plot of the instantaneous concentration of Tracer Q in the 3 unknown compartments of the model and a plot that is equivalent to what the PET scanner would measure if it measured in infinitely short time frames and Tracer Q was labeled with an EXTREMELY slowly decaying radio-isotope.

***Please label the four curves on the lines at the right of the figure.
Concentration of tracer (pmol/ml)

Figure 4

(C)

note: total is what the PET scanner would measure if there were not decay. Red is the sum of blue green and black. It must be infinitely fine time frames to give a continuous curve.
You are a young scientist studying bone disease. You are interested in one disease (Disease 1) which is hypothesized to be caused by insufficient blood flow to bone and another condition (Disease 2) which is believed to be caused by inadequate numbers of Receptor Q molecules to be produced by bone cells.

You have access to all three types of subjects who are either
- normal
- have Disease 1
- have Disease 2

On a limited budget you can only afford to inject each subject once with labeled Tracer Q and to acquire two separate three-minute scans to investigate both diseases. (Radiology charges by the number of time-frames you acquire.)

***Where in time should you acquire scan 1 to accomplish your goals of comparing normals to Disease 1 patients and scan 2 to compare normals to Disease 2 patients. ***Why?

Scan in first 3 minutes since most of PET signal is due to Free which depends on $K_1^*C_a$ where $K_1 = FE$

and Scan 2 in late time where most of PET is dependent on Bound which depends on $k_3$.

(D) ***A new version of Tracer Q (‘Tracer Super Q’) is very sticky – it binds extremely tightly to the Q Receptor (once bound, there is a small likelihood of dissociation in one hour). If you only scan for one hour no matter how you analyze your data, what can you do to simplify the model in Figure 3?

Assume that Super Q is irreversible in the time course of the scan and thus we can set $k_4 = 0$
(20 points) Part IV. Creating a new model  

A new tracer is made from Trident gum ($^{11}$C-tri-g). It diffuses freely out of the vasculature and into the extracellular space. It is transported into the cell via a transporter that normally exists in great excess. The $^{11}$C-tri-g tracer has the following special property: once inside heart cells, it binds to an enzyme and a co-enzyme. Only when it binds to both entities can it be phosphorylated and trapped in the cell.

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**Key**

- transporter
- tracer
- phosphorylated tracer
- co-enzyme
- enzyme

**Figure 5**
(A) *** Draw the compartmental model for the uptake from the blood and retention by heart cells of the Trident gum tracer.

\[
\begin{array}{c}
\text{Plasma} \\
\text{Free} \\
\text{Bound}
\end{array}
\]

(B) *** If the transport of $^{11}$C-tri-g out of the blood and into the cells is not near saturation, then write the mass balance equations for the free $^{11}$C-tri-g in the cells and for the production of phosphorylated $^{11}$C-tri-g-PO$_4$. Please write the balance on the free and bound states to reflect that either enzyme or co-enzyme might be in short supply.

\[
\begin{align*}
\frac{dF(t)}{dt} &= K_1 C_p(t) - k_2 F(t) - k_{on} [\text{Available Enzyme}][\text{Available Co-Enzyme}]F(t) \\
\frac{dB(t)}{dt} &= k_{on} [\text{Available Enzyme}][\text{Available Co-Enzyme}]F(t)
\end{align*}
\]

“tri-molecular binding!”

(C) *** If the transporter on the cell surface is a limiting factor in $^{11}$C-tri-g uptake, but the co-enzyme and enzyme are both in great excess, then rewrite the balance on the free $^{11}$C-tri-g concentration.

\[
\frac{dF(t)}{dt} = K_1 [\text{Available transporter}]C_p(t) - k_2 F(t) - k_3 F(t)
\]
(D) *** We want to model the uptake of $^{11}$C-tri-g but we cannot convince any students to volunteer to have their arteries punctured while being scanned. Dr D----, a cell physiologist has observed that there are clumps of cells in the heart that have the enzyme but no co-enzyme for phosphorylating $^{11}$C-tri-g. Can we use this to our advantage? Why or why not.

Yes. We can use the special clumps of heart tissue (if we can image them) as reference regions. Even though they have enzyme, there will be no binding process ($k_3=0$) since there is absolutely NO available co-enzyme.

As a reference region, we can rearrange

$$\frac{dF(t)}{dt} = K_1 C_P(t) - k_2 F(t)$$

to get an expression for $C_p(t)$ in terms of the free concentration in the reference region. (Then, all we need to do is assume that the PET signal is entirely Free—that is, we must neglect the blood volume fraction. This might be tricky in the heart since we image tissue close to a hot pool of blood—but I never specified where the special clumps were. Assume they are far from the endocardium.)