Determining Alterations in the Serotonin Uptake and Synthesis in the Gastrointestinal Tract Based on a Model of Convective Transport and Tryptophan Conversion*

Jason Cain,[1] Nathan Harms,[1] Marissa Puzan,[1] and Richard West[1,†]

[1]*Department of Chemical Engineering, Northeastern University, Boston, MA 02115*

Production and uptake of serotonin within the gastrointestinal tract (GI) depend on a variety of factors, including intestinal health, tryptophan intake, and the host microbiome. To study the effects of these factors on the host's uptake of serotonin, a kinetic model was created, considering the transport, diffusion, and production or consumption of both serotonin and tryptophan within the GI. This model allows for the study of the impact of multiple variables on host serotonin levels in the GI, providing new targets for both gastrointestinal maladies and psychological conditions related to low serotonin levels.

Keywords: Python, Kinetic Modeling, Serotonin, Gastrointestinal, Tryptophan.

I. INTRODUCTION

Serotonin (5-hydroxytryptamine, or 5-HT) is a major neurotransmitter within the central nervous system and the brain, with alterations in serotonin signaling implicated in many psychological disorders including anxiety, depression, and autism. Up to 95% of the body's total serotonin is not found within the brain, but is located within the gastrointestinal tract, where it regulates intestinal motility and peptide secretion [1] [2]. Dysfunction in serotonin production within the gut is linked to gastrointestinal disorders such as irritable bowel syndrome and colitis.

Synthesis of serotonin from dietary tryptophan occurs mainly by tryptophan hydroxylase 1 (TPH1) found on the apical surface of enterocromaffin cells and the brush borders of enterocytes located within the intestinal epithelium, which directly interacts with intestinal contents and regulates the uptake of nutrients within the intestinal environment. The specialized enterocromaffin cells release serotonin in response to mechanical stimuli, which triggers muscle contraction and secretion of vasoactive intestinal peptide (VIP)

* Project for Computational Modeling for Chemical Engineers, CHME 5137, Fall 2016

† Corresponding author, Dr. Richard West, Department of Chemical Engineering, Northeastern University, 360 Huntington Avenue, Boston, MA, 01225. r.west@northeastern.edu

for downstream signaling and regulation of satiety. As serotonin is absorbed by the intestine, some is taken up by enteric neurons and glia, and some released into the bloodstream, where it is taken up by platelets and transported throughout the body.

Recent studies have also shown the some species from the normal gastrointestinal microbiota are able to synthesize serotonin and other neurotransmitters, enabling them to interact with the host's own neural signaling pathways [3] and providing a pathway for communication between the microbiota and the brain in Figure 7 [4] [5].

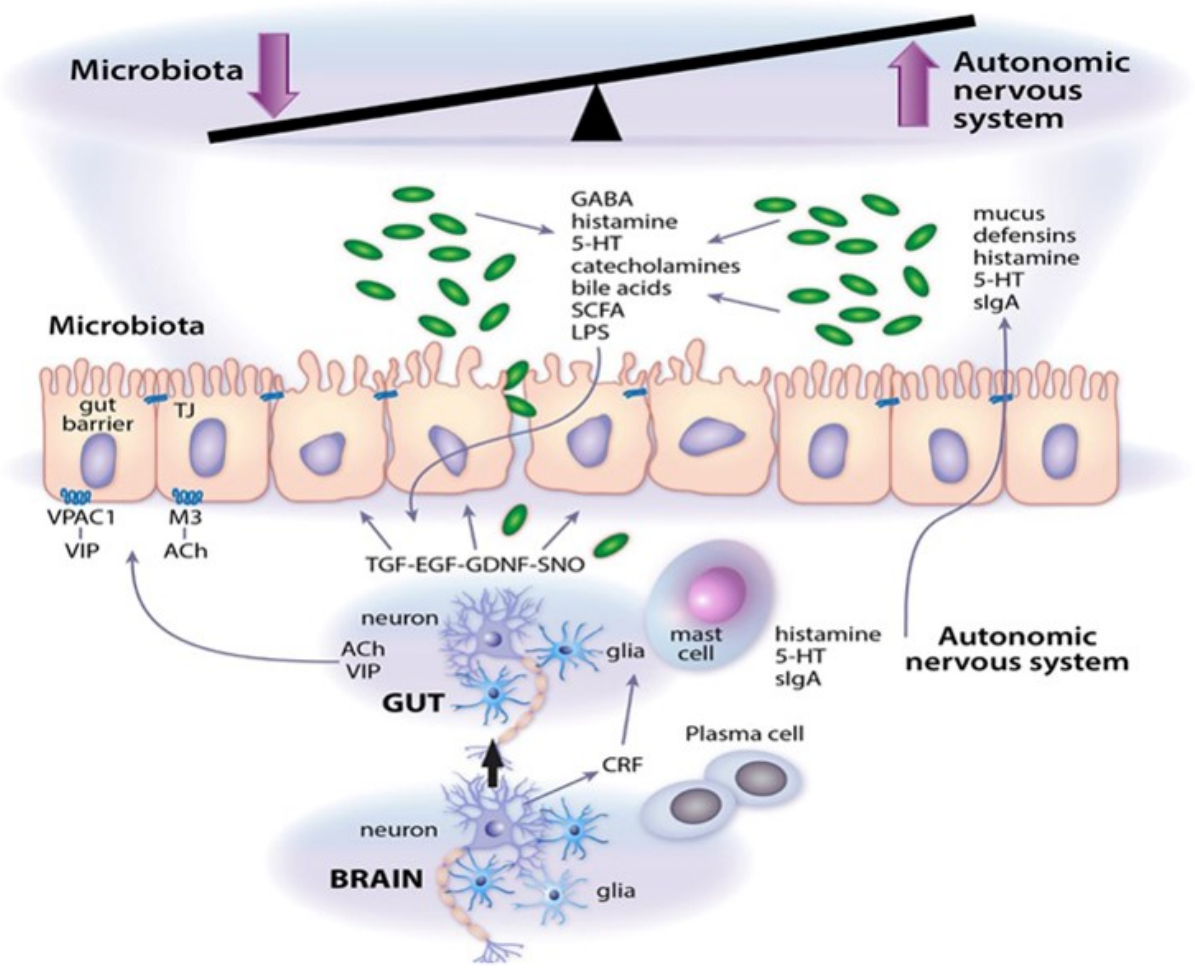


Fig. 1: Serotonin is Located throughout the Intestinal Environment

The production and uptake of serotonin in the GI depends on a number of variables, including epithelial health and permeability, tryptophan intake, enterocromaffin cell number, and microbiota composition, as some species of yeast and bacteria are able to synthesize serotonin and its precursors. Limited resources are available that consider all these factors and their relation to total serotonin uptake, although serotonin deficits can dramatically affect mental health and behavior, creating a need for a computational model of

this system to allow for rapid study of specific alterations in the intestinal environment.

II. THEORY

In order to model this complex process, the whole of the GI was considered a semipermeable tube, with constant laminar flow profile over its entire length. Iterations over both the length (in z) and radius (in r) allowed for determination of concentration profiles in both axial and radial dimensions over time, considering convection, diffusion, conversion of tryptophan to serotonin both in the bulk and at the wall, and uptake of serotonin at the intestinal wall. Figure 2 shows the model system, in cylindrical coordinates, with divisions over both the length and radius.

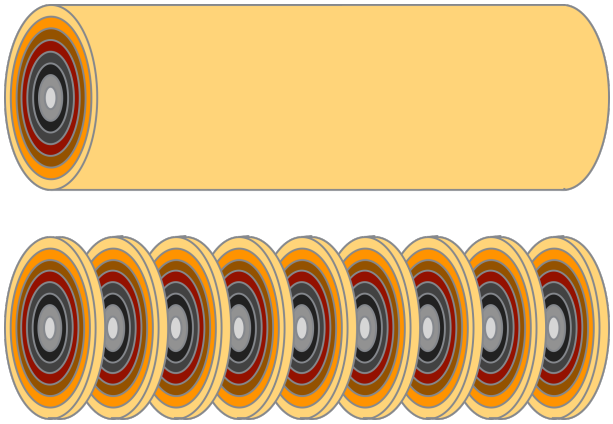


Fig. 2: Intestinal Divisions in r and z

The subdivisions over both the length, Δz, and over the radius, Δr Figure 3, allow for consideration of both a laminar velocity profile and diffusion in both r and z directions, providing more realistic concentration profiles both at the wall and the center of the tube. Previous studies have shown a preferential fit of a laminar flow model versus a plug flow model to experimental data [6] [7].

Three major processes have been considered for this system:

Molecular Transport: Both convective and diffusive transport of Tryptophan, 5-Hydroxytryptophan (5-HTP), and serotonin (5-Hydroxytryptamine, or 5-HT) occur along the length of the intestine. Assuming convection occurs in only z and a low Reynolds number, a hyperbolic laminar flow profile was applied. Both axial and radial diffusion occur along concentration gradients in the system.

Conversion of Tryptophan to Serotonin: Within the body, tryptophan is converted to serotonin in a two

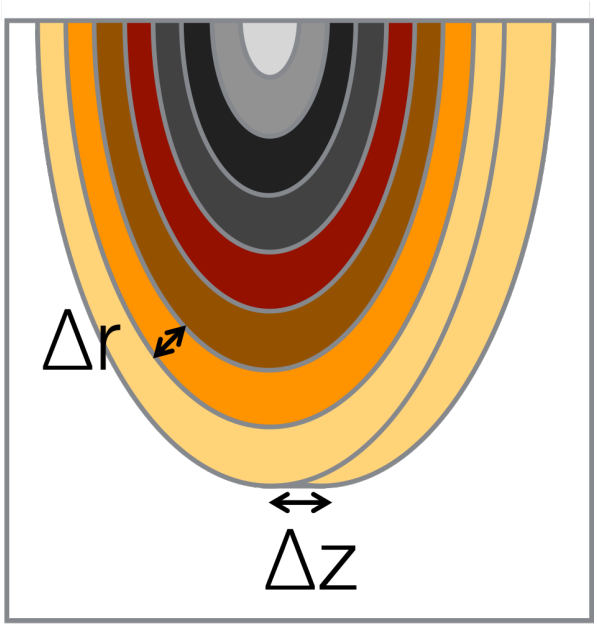


Fig. 3: Sample Intestinal Divisions, Δz and Δr, in Cylindrical Coordinates

step enzymatic process (Figure 4). First, in the rate limiting step, tryptophan is converted to 5-hydroxytryptophan by TPH1, tryptophan hydroxylase 1, found in enterocromaffin and epithelial cells. Second, 5-HTP is converted to 5-HT by DOPA-decarboxylase, a general amino acid decarboxylase, which occurs rapidly. Some species of bacteria are also able to convert tryptophan to serotonin, although the mechanism is largely unknown [3]. A two step, Michaelis-Menten equation was fit to experimental data to allow for consideration of bacterial conversion.

Uptake at the Intestinal Epithelium: Permeability through the intestinal epithelium occurs through serotonin transporter (SERT) [8]. In this system, the intestinal epithelium was considered a semipermeable membrane, with effective permeability determining rate of serotonin uptake. The model assumes no build up of serotonin concentration within the tissue; serotonin is immediately take up by enteric neurons or glia or immediately diffuses into the bloodstream. [5]

Each process will be considered in more detail in the following methods.

III. METHODS

A. Mass Balance

In this system, transport of tryptophan, 5-hydroxy-L-tryptophan (5HTP) and serotonin was modeled via diffusion, convection, and reaction. A traditional mass balance on each subsection of intestine was used to develop the differential equation. Similar approaches have assumed that the mass balance is at a steady state condition [6].

$$C_i\Big|_{t+\Delta t} - C_i\Big|_{t} = -D_i\nabla^2 C_i\Delta t + \frac{v_z}{\Delta z}[C_i\Big|_{z+\Delta z} - C_i\Big|_{z}]\Delta t + r_i\Delta t \tag{1}$$

Next, dividing by Δt and taking the limit as Δt and Δz approach 0, gives the final differential equation.

$$\frac{\partial C_i}{\partial t} = \underbrace{-D_i\nabla^2 C_i}_{\text{Diffusion}} + \overbrace{v_z\frac{\partial C_i}{\partial z}}^{\text{Convection}} + \underbrace{r_i}_{\text{Reaction Kinetics}} \tag{2}$$

This equation is broken into three distinct parts: diffusion, convection, and reaction kinetics. They will be described more thoroughly in subsequent subsections.

B. Diffusion

Diffusion of all species will follow Fick's second law (equation 3) where D_i is the diffusion coefficient for species i, and $\nabla^2 C_i$ is the concentration gradient in all directions.

$$\frac{\partial C_i}{\partial t}_{Diffusion} = -D_i\nabla^2 C_i \tag{3}$$

For the purpose of this model, diffusion will only be considered in the r and z directions so the concentration gradient is expanded as follows.

$$\frac{\partial C_i}{\partial t}_{Diffusion} = -D_i[\frac{\partial^2 C_i}{\partial z^2} + \frac{1}{r}\frac{\partial}{\partial r}(r\frac{\partial C_i}{\partial r})] \tag{4}$$

Further expansion yields the following equation.

$$\frac{\partial C_i}{\partial t}_{Diffusion} = -D_i[\frac{\partial^2 C_i}{\partial z^2} + \frac{\partial^2 C_i}{\partial r^2} + \frac{1}{r}\frac{\partial C_i}{\partial r}] \tag{5}$$

First and second derivatives of concentration in the z and r direction were estimated using the concentration differential between neighboring ring sections.

Diffusion constants through intestinal mucus were estimated using data for small molecule drug diffusivites from Larhed et. al [9]; a model molecule was chosen with similar size and functional groups to tryptophan and serotonin. This relationship is given in equation 6 and the tabulated diffusion coefficients are given in the following table.

$$D \cdot 10^6 \cdot MW^{(1/3)} = 11 \pm 2 \tag{6}$$

Table 1: Calculated Diffusion Coefficients of Species

Parameter	Value	Units
D_{Tryp}	$5.386 \cdot 10^{-8}$	m^2/s
D_{5HTP}	$4.995 \cdot 10^{-8}$	m^2/s
D_{Ser}	$6.242 \cdot 10^{-8}$	m^2/s

C. Convection

In order to determine convective mass transfer, the mass flow rates of species of interest were used to develop a mass balance. It was assumed that the flow was incompressible. The parameters in the following equation are as follows: ΔV is the volume of the ring subsection, F_i is the mass flow of species i, A is the cross-sectional area of the ring subsection, and v_i is the velocity of the fluid in the z-direction.

$$\Delta V \frac{\partial C_i}{\partial t}_{Convection} = F_i\Big|_z - F_i\Big|_{z+\Delta z} = A v_z (C_i\Big|_z - C_i\Big|_{z+\Delta z}) \tag{7}$$

$$\frac{A}{\Delta V} = \frac{A}{A\Delta z} = \frac{1}{\Delta z} \tag{8}$$

Substitution of equation 8 into equation 7 yield the following equation.

$$\frac{\partial C_i}{\partial t}_{Convection} = \frac{v_z}{\Delta z}(C_i\Big|_z - C_i\Big|_{z+\Delta z}) \tag{9}$$

And taking the limit as Δz approaches 0 creates the following differential equation:

$$\frac{\partial C_i}{\partial t}_{Convection} = v_z \frac{\partial C_i}{\partial z} \tag{10}$$

The velocity in the z-direction was determined assuming a no slip boundary condition at the wall of the intestine and the assumption that the maximum velocity will occur in the center of the intestine, following a laminar flow profile. These two assumptions were combined to generate the following relationship:

$$v_z = v_{z,max}(1 - (\frac{r}{R})^2) \tag{11}$$

In the previous equation, $v_{z,max}$ is the maximum velocity of fluid, r is the radius of the ring of interest and R is the maximum radius. And combining these two equations yields the final differential equation for convection.

$$\frac{\partial C_i}{\partial t}_{Convection} = v_{z,max}(1 - (\frac{r}{R})^2)\frac{\partial C_i}{\partial z} \tag{12}$$

D. Reaction Kinetics

The reaction that occurs in this system is a two step process as described in Figure 4.

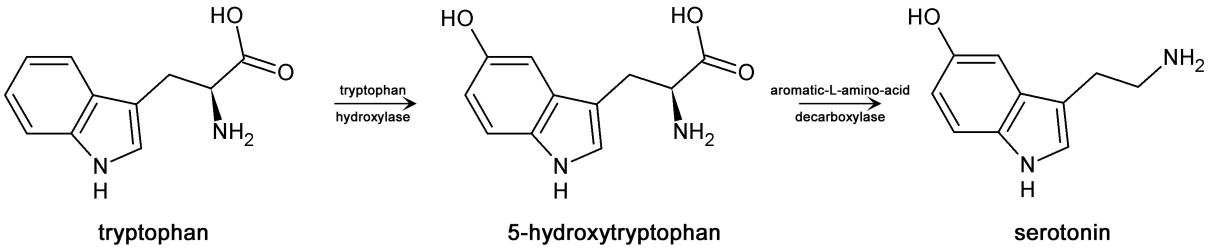


Fig. 4: Conversion of Dietary Tryptophan to Serotonin Occurs in a Two-Step Enzymatic Process

Due to the fact that this is a biological system, Michaelis-Menten (M-M) kinetics were assumed for both reactions in the two step process. The traditional M-M rate equation is given in equation 13. In the M-M rate equations, V_{max} is the maximum rate and K_m is the Michaelis constant. These are both intrinsic properties of the enzymes that catalyze the reaction.

$$r_a = \frac{V_{max} \cdot C_a}{K_m + C_a} \tag{13}$$

Because tryptophan consumption is achieved by a tryptophan-specific enzyme (tryptophan hydroxylase), this reaction rate has no competitive inhibition and the rate can be expressed using the traditional

M-M kinetics as shown below.

$$r_{tryp} = -\frac{V_{max,1} \cdot C_{tryp}}{K_{m,1} + C_{tryp}} \tag{14}$$

The rate of production of the intermediate 5-HTP is the combined rate of consumption of trypotphan and the rate of production of serotonin. This is displayed in equation 15.

$$r_{5HTP} = \underbrace{\frac{V_{max,1} \cdot C_{tryp}}{K_{m,1} + C_{tryp}}}_{\text{5-HTP Production}} - \overbrace{\frac{V_{max,2} \cdot C_{5HTP}}{K_{m,2} + C_{5HTP}}}^{\text{5-HTP Consumption}} \tag{15}$$

Finally, the rate of serotonin production is negative of the rate of consumption of 5-HTP, so, the rate of serotonin production is as follows.

$$r_{ser} = \frac{V_{max,2} \cdot C_{5HTP}}{K_{m,2} + C_{5HTP}} \tag{16}$$

Kinetic parameters for the M-M equations were fitted to experimental data provided by Park et. al. using the `scipy.optimize` feature. The values are listed in the following table.

Table 2: Determined Michaelis-Menten Parameters

Parameter	Value	Units
$V_{max,1}$	0.1868	mM/h
$K_{m,1}$	1.4353	mM
$V_{max,2}$	9.9770	mM/h
$K_{m,2}$	2.3743	mM

E. Boundary Conditions at the Intestinal Wall

Molecular transport at the intestinal wall differs from that in the bulk, as wall permeability has to be considered at this boundary. The mass balance has to be adjusted to equation 17. This new mass balance accurately reflects the no slip boundary condition at the wall by eliminating the convective mass transfer term from the equation.

$$\frac{\partial C_i}{\partial t} = -D_i \nabla^2 C_i + r_i^* \tag{17}$$

The diffusion will remain the same in the z-direction; however, the r-direction diffusion will substituted with permeability through a membrane as showing in the following equation.

$$\frac{\partial C_i}{\partial r} = \frac{P_{wall}}{D_i} C_{wall} \tag{18}$$

Where P_{wall} is the permeability of species i through the intestinal wall, D_i is the diffusivity of species i, and C_{wall} is the concentration of species i at the wall. This equation substitutes into the diffusion equation as follows:

$$\frac{\partial C_i}{\partial t}_{Diffusion} = -D_i \frac{\partial^2 C_i}{\partial z^2} + \frac{1}{r}\frac{\partial}{\partial r}(r P_{wall} C_{wall}) \tag{19}$$

$$\frac{\partial C_i}{\partial t}_{Diffusion} = -D_i \frac{\partial^2 C_i}{\partial z^2} + P_{wall}\frac{C_{wall}}{r} \tag{20}$$

Next, the reaction kinetics will follow a similar M-M type reaction rate; however, the parameters (V_{max}, K_m, etc.) will change because the concentration of bacteria facilitating the reaction is different at the wall compared to the bulk. These parameters were provided by [10] [11]. The resultant parameters are found in the following table.

Table 3: Reported Michaelis-Menten Parameters at the Intestinal Wall

Parameter	Value	Units
$V_{max,1}$	$7.56 \cdot 10^1 4$	$\frac{mM}{h}$
$K_{m,1}$	$6.0 \cdot 10^4$	mM
$V_{max,2}$	7.14	$\frac{mM}{h}$
$K_{m,2}$	$1.19 \cdot 10^2$	mM

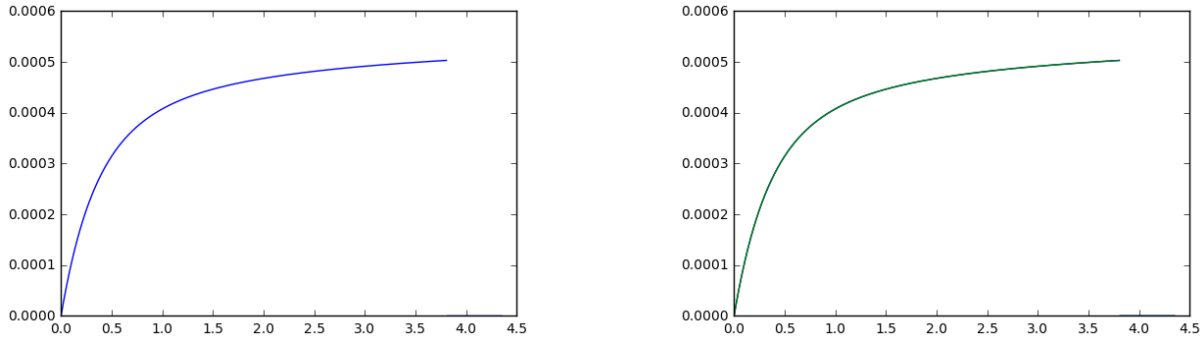
IV. RESULTS

The goal of this model was to measure the overall uptake of serotonin through an initial spike of tryptophan and serotonin. This was initialized by setting the concentration in the first z section to the initial concentration of interest, and then running the simulation.

Serotonin uptake was calculated using the following boundary condition:

$$\delta\Big|_{t=t+\Delta t} = \delta\Big|_{t=t} + P_{wall} \cdot \sum_{z=1} 2\pi R \Delta z \cdot C_{Ser}\Big|_{r=R} \tag{21}$$

Where δ is the serotonin uptake at different time points, P_{wall} is the permibility of serotonin at the wall, R is the total radius, Δz is the length of the subsection and C_{Ser} is the concentration of serotonin. Equation 21 was used to generate the following plots.



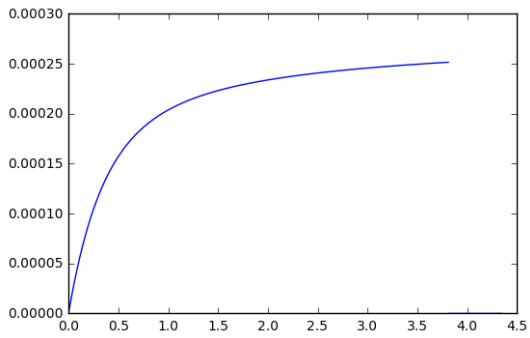
(a) Tryptophan 1mM, Serotonin 1mM

(b) Tryptophan 2mM, Serotonin 1mM

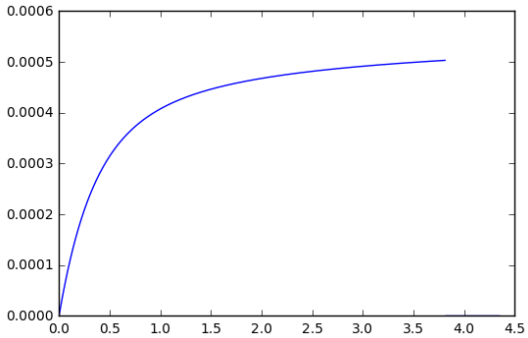
Fig. 5: Total Serotonin Uptake (mM) over Time (h) is not Affected by Initial Starting Concentration of Tryptophan

Total serotonin uptake along the intestine was determined given initial starting concentrations of serotonin and tryptophan at the entrance, simulating food intake. First, changes in initial tryptophan concentration were studied. Figure 5 compares the total serotonin uptake for initial tryptophan concentrations of 1 mM and 2 mM at the entrance of the intestine, with serotonin concentration held constant at 1 mM. The plots show that total serotonin uptake over the 4 hours of simulated digestion is the same, approaching 0.0005 millimoles.

Next, changes in total serotonin uptake based on initial serotonin concentration were determined. Figure 6 shows that the total uptake for an initial concentration of 0.5 mM serotonin is 0.00025 millimoles, and total uptake with an initial concentration of 1 mM serotonin is 0.0005 millimoles, a 100% increase in uptake with due to a 100% increase in the starting concentration of serotonin. The direct relationship between starting serotonin concentration and serotonin uptake combined with the lack of uptake when initial serotonin uptake is set to zero (Figure 7) lead to the conclusion that the reactions modeled in this system are not taking place, likely due to inaccurate reaction rate parameters.



(a) Tryptophan 0.5 mM, Serotonin 0.5 mM



(b) Tryptophan 0.5 mM, Serotonin 1mM

Fig. 6: Total Serotonin Uptake (mM) over Time (h) Depends on Initial Starting Concentration of Serotonin

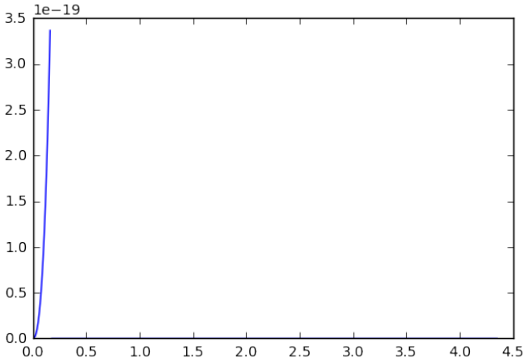


Fig. 7: Without an Initial Concentration of Serotonin, No Serotonin Uptake Occurs

V. RECOMMENDATIONS

The model should be validated with data with experiments determining the total uptake of serotonin as a function of the concentrations of both tryptophan and serotonin entering the intestine.

VI. LIMITATIONS OF THIS MODEL

The model developed here provides a method to study the intestinal uptake of a molecule, considering both molecular flux and production within the GI tract, which could feasibly be applied to any small molecule of interest; however, certain limitations exist due to limited data availability and necessary estimation of key parameters for calculating molecular transport and production. Limitations of the proposed serotonin uptake model occur primarily as a result of the lack of reaction kinetic data for the tryptophan to

serotonin conversion. The Michaelis-Menten constants that defined the reaction were loosely estimated. Without additional data for the reaction within the bulk media as well as at the wall, the rate laws can not be properly integrated. The current estimates for the kinetics parameters determined the reaction was not significant in the calculation of serotonin uptake.

A. Parameter Estimation

To investigate the gastrointestinal uptake of serotonin, several parameters, including diffusivities, Michaelis-Menten parameters, and permeabilities were estimated from published data and assumed constant throughout the system. Diffusivities were estimated for serotonin, tryptophan, and 5-HTP in intestinal mucus, which readily applies at the wall, but may prove inaccurate towards the intestinal center. Diffusivities within the bulk would depend both on intestinal contents and location along the length of the intestine, neither of which were accounted for in this model. Intestinal permeability is highly dependent of the overall health of the intestine, which is partially regulated by serotonin concentration [12]. Mechanisms behind this interaction are largely unknown, thus serotonin's alteration of intestinal permeability has not been considered.

B. Reaction Kinetics

Bulk Reaction: Only recently has research shown that specific strains of gastrointestinal microbes are able to produce serotonin [3]. Rates of either production, or possibly consumption, as other GI neurotransmitters, like γ-Aminobutyric acid (GABA) and dopamine are consumed by microbes [13], depends both on overall and species specific microbe concentrations.

Wall Reaction: The enzymatic conversion of tryptophan to serotonin was modeled as a two step, Michaelis Menten type kinetic reaction. The parameters used for v_{max} and K_m for each reaction (via TPH1 and DDC) were taken from literature. Again, due to limited availability of data, enzymatic activity was estimated from values for both TPH1 and DDC, leading to the significant error in these values.

VII. CONCLUSIONS

From this model, serotonin uptake against time plots were generated using an overall mass balance, as derived from 2 and detailed in the methods section.

Initially, a method using a steady state mass balance modeled by Amidon et al. [6] was used to model the intestinal tract, which calculated the total uptake based on the difference in concentration from the leading edge of the intestine through the length. This method would describe the system over the entirety of the intestine, but was not able to describe the system at differential time intervals to calculate simultaneous reaction rates. Secondly, Euler's method was attempted to solve this differential equation, in equation 2. The first and second derivatives were estimated and used in conjunction with the mass balance to determine the concentrations of tryptophan, 5-HTP and serotonin through out each r and z subsection at each time point. However, this method was not able solve this stiff differential equation. The rapid diffusion of the system based on the initial concentrations led to significant changes during the initial time points.

Because of this, `scipy.odeint` was finally used to attempt to solve this differential equation. However, because of the large number of changes happening in a short amount of time, `odeint` required very small time steps to converge on a solution. This resulted in a solution with a very costly computation time. Possible ways to address this issue is to look at how alternative differential equation solvers can be integrated into the model.

In order to create a more accurate model, the diffusivities and permeabilities of tryptophan, 5-HTP, and serotonin should be studied further. This will allow for a more accurate estimation of total serotonin uptake.

VIII. REFERENCES

[1] Sylvie Ramboz, Ronald Oosting, Djamel Aït Amara, Hank F Kung, Pierre Blier, Monica Mendelsohn, J John Mann, Dani Brunner, and René Hen. Serotonin receptor 1a knockout: an animal model of anxiety-related disorder. *Proceedings of the National Academy of Sciences*, 95(24):14476–14481, 1998.

[2] Joel C Bornstein. Serotonin in the gut: what does it do? *Frontiers in neuroscience*, 6:16, 2012.

[3] VA Shishov, TA Kirovskaya, VS Kudrin, and AV Oleskin. Amine neuromediators, their precursors, and oxida-

tion products in the culture of escherichia coli k-12. *Applied biochemistry and microbiology*, 45(5):494–497, 2009.

[4] Emeran A Mayer, Rob Knight, Sarkis K Mazmanian, John F Cryan, and Kirsten Tillisch. Gut microbes and the brain: paradigm shift in neuroscience. *The Journal of Neuroscience*, 34(46):15490–15496, 2014.

[5] Doe-Young Kim and Michael Camilleri. Serotonin: a mediator of the brain-gut connection. *The American journal of gastroenterology*, 95(10):2698, 2000.

[6] GL Amidon, J Kou, RL Elliott, and EN Lightfoot. Analysis of models for determining intestinal wall permeabilities. *Journal of pharmaceutical sciences*, 69(12):1369–1373, 1980.

[7] D Winne. Unstirred layer, source of biased michaelis constant in membrane transport. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 298(1):27–31, 1973.

[8] Zhishan Li, Alcmène Chalazonitis, Yung-yu Huang, J John Mann, Kara Gross Margolis, Qi Melissa Yang, Dolly O Kim, Francine Côté, Jacques Mallet, and Michael D Gershon. Essential roles of enteric neuronal serotonin in gastrointestinal motility and the development/survival of enteric dopaminergic neurons. *The Journal of Neuroscience*, 31(24):8998–9009, 2011.

[9] Agneta Wikman Larhed, Per Artursson, and Erik Björk. The influence of intestinal mucus components on the diffusion of drugs. *Pharmaceutical research*, 15(1):66–71, 1998.

[10] Fuchun Jing and Jun Zhang. Metabolic kinetics of 5-hydroxytryptamine and the research targets of functional gastrointestinal disorders. *Digestive diseases and sciences*, 59(11):2642–2648, 2014.

[11] Anne-Kristin Stavrum, Ines Heiland, Stefan Schuster, Pål Puntervoll, and Mathias Ziegler. Model of tryptophan metabolism, readily scalable using tissue-specific gene expression data. *Journal of Biological Chemistry*, 288(48):34555–34566, 2013.

[12] Michael D Crowell. Role of serotonin in the pathophysiology of the irritable bowel syndrome. *British journal of pharmacology*, 141(8):1285–1293, 2004.

[13] E Barrett, RP Ross, PW O'Toole, GF Fitzgerald, and C Stanton. γ-aminobutyric acid production by culturable bacteria from the human intestine. *Journal of applied microbiology*, 113(2):411–417, 2012.