Computational Model of Urea and Ammonia in the Blood and the Gastric Juice

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1 The Code

The model uses the *odeint* function from the *scipy.integrate* package to run the differential equations. The simulation takes in its time parameters as *np.linspace* so the length and resolution can be adjusted easily. The simulation also takes in glomerular filtration rate (GFR) as a parameter so different stages of kidney disease can be modeled.

2 The Biological Model

This section will describe my understanding of the nitrogen cycle in the body, as well as the interaction between the blood, gastrointestinal tract (GIT), kidney, and liver. Protein is eaten and enters the GIT, where is it broken down into amino acids, ammonia, and other products. Moreover, urea also diffuses into the GIT and is broken down into ammonia by urease-containing bacteria. Some of this ammonia and other products are absorbed into the portal vein, the blood vessel leading directly from the GIT to the liver. A significant amount of the ammonia in the GIT is used by bacteria to produce more products, such as amino acids. In the liver, urea is produced from ammonia and the metabolism of amino acids and enters the systemic circulation. Still, not all ammonia is converted to urea so some ammonia enters the systemic circulation from the GIT. The liver also filters the blood in systemic circulation, so the systemic ammonia is constantly being converted to urea.

In healthy patients, the kidneys filter out urea to maintain Nitrogen balance. In patients with chronic kidney disease (CKD), however, the kidneys are not able to filter out urea at an efficient-enough rate so the level of urea in the systemic circulation increases, a condition known as uremia.

This model seeks to simulate the effects of a urease treatment that will break down urea into ammonia. As a result, in patients with CKD, more nitrogen will be excreted as ammonia and other nitrogenous products than would be by the kidneys in the form of urea. In essence, the treatment would transform Nitrogen into a form that is more easily excreted by the body - through ammonia and

bacterial products in the feces - than urea by the diseased kidneys. Simulating this treatment will allow us to gain insight into the effects of the treatment on blood urea levels and of excess ammonia produced by the treatment.

3 Urea Transport

3.1 Assumptions

- **Assumption:** The kinetics of urea transport across the lining of the GIT are the same as those across the red blood cell (RBC) membrane.
- **Justification:** Urea diffuses across the cell membrane through passive transport by urea transporters (UT) and other transport proteins. Therefore, a simple diffusion equation will not accurately model urea transport and a passive transport equation is needed. Jesper Brahm analyzed urea transport across the RBC membrane and derived constants for a Michaelis-Menten (M-M) equation to describe urea transport as well as permeability constants at different concentrations of urea. At a urea concentration of 50 mM, Brahm calculated a permeability constant of approximately $2 * 10^{-}4cms^{-}1$ for urea transport across RBC membranes and Fagerholm et al. calculated, at a urea concentration of 50 mM, a permeability constant of $3.0 * 10^{-}4cms^{-}1$ for urea transport across the small intestine by perfusing an isotonic solution. Because the permeability constants at 50 mM urea are roughly equal, it is appropriate to use the Michaelis-Menten constants that Brahm found for urea transport across RBC membranes for urea transport across the small intestine.
- **Assumption:** There are enough UTs to faciliate urea transport both from the blood into the GIT and vice versa without the transport rate in one direction affecting the transport rate in the other.
- **Justification:** In his studies, Brahm found that $K_{\frac{1}{2}} = 334\frac{mmol}{L}$. The urea concentration in patients with end-stage renal disease is $50\ \frac{mmol}{L}$, which is much less than $K_{\frac{1}{2}}$. Therefore, it is safe to assume that the maximum rate of transport will not become lower with bidirectional flow as opposed to unidirectional flow, for which Brahm derived the (M-M) constants.
- **Assumption:** The surface area of cells touching the blood is the same as the surface area of the cells touching the GIT fluid, or the inner surface area of the GIT.
- **Assumption:** The volume of gastric juice in the stomach and the volume of blood in the body is constant.
- **Assumption:** The patient has proper liver function

With these assumptions, I am able to model urea transport from the blood into the GIT fluid and vice versa. Thus, the equations I used to describe the millimolar flux of urea from the blood to the GIT fluid, and vice versa, were:

$$J_{blood \to GIT} = \frac{J_{max} U_{blood}}{K_m + U_{blood}} \tag{1}$$

$$J_{GIT \to blood} = \frac{J_{max} U_{GIT}}{K_m + U_{GIT}} \tag{2}$$

where

$$J_{max} = 8.3 * 10^{-5} \, \frac{mmol}{cm^2 * sec}$$

$$K_m = 345 \, \frac{mmol}{L}$$

$$[J] = \frac{mmols}{cm^2 * sec}$$

$$[K] = \frac{mmol}{L}$$

$$[U] = \frac{mmol}{L}$$

To arrive at the final equations describing urea movement across the GIT membrane, I simply multiplied each of the above equations by the inner surface area of the GIT (CITE):

$$\boxed{V_{blood \to GIT} = SA * \frac{J_{max} U_{blood}}{K_m + U_{blood}} \, \frac{mmols}{s}} \tag{3}$$

$$\boxed{V_{GIT \to blood} = SA * \frac{J_{max} U_{GIT}}{K_m + U_{GIT}} \, \frac{mmols}{s}} \tag{4}$$

where

$$SA = 32000 \, cm^2$$

4 Urea Production

According to CITE, urea production is a function of grams of protein eaten per day (PPD). Approximately 16% of the mass of protein is Nitrogen and there are two Nitrogen molecules in urea. Therefore:

$$Mass \, N \, per \, day = 0.16 * PPD \tag{5}$$

$$Mmols \, Urea \, per \, day = Mass \, N \, per \, day * \frac{1000 \, mmol \, Urea}{28 \, g \, N} \tag{6}$$

$$V_{urea \, production} = Mmols \, Urea \, per \, day * \frac{1 \, day}{86,400 \, seconds} \tag{7}$$

In short:

$$\boxed{V_{urea \, production} = 0.00006614 * PPD \, \frac{mmols}{s}} \tag{8}$$

5 Urea Excretion

The GFR, $\frac{mL}{min}$ of a patient is a measure of how much blood the kidneys filter per unit of time. In healthy patients, the GFR is above 90 $\frac{mL}{min}$, but in patients with CKD, it decreases. According to (CITE), In normal individuals, the urea clearance ranges from as high as 65% of GFR during diuresis to as low as 35% of GFR during antidiuresis. In patients with GFR less than 20 $\frac{mL}{min*1.73\,m^2}$ (1.73 m^2 is the average surface area of a human), the urea clearance ranges from 70% to 90% of GFR and is not influenced greatly by the state of diuresis."

In the model, I assumed that at a GFR level at or above 20 urea clearance is 40% of GFR, and below twenty, urea clearance increases to 80%. This is a cool example of how the body can change its functions in response to different conditions. So, urea clearance as a function of GFR is:

$$Urea\,Clearance\,(GFR) = \begin{cases} 0.4 & GFR \geq 20 \\ 0.8 & GFR < 20 \end{cases}$$

and the rate of urea clearance, as a function of GFR, is:

$$L_{Urea\,Clearance} = GFR * Urea\,Clearance\,(GFR) * \frac{1\,L}{1000\,mL} * \frac{1\,min}{60\,s} \tag{9}$$

where

$$[L] = \frac{L}{sec}$$

When $L_{Urea\,Clearance}$ is multiplied by the concentration of urea in the blood in $\frac{mmol}{L}$, the units of the product become $\frac{mmols}{s}$, as desired. Thus:

$$\boxed{V_{Urea\,Excretion} = U_{Blood} * L_{Urea\,Clearance}} \tag{10}$$

where

$$[V] = \frac{mmols}{s}$$

6 Urea Breakdown by Bacteria in the GIT

6.1 Assumptions

- **Assumption:** Under basal conditions, urea concentrations in the GIT and in the blood are equal.
- **Justification:** In (CITE), it is written, "Because of high small-intestine urea permeability, the luminal intestinal urea concentration is approximately equal to plasma urea (3.2 mM)." Therefore, it is appropriate to assume that under basal conditions, the urea concentrations in the blood and GIT are equal.
- **Assumption:** The rate of urea breakdown by enteric bacteria at a urea concentration of 3.2 mM is saturated and is the maximum rate of breakdown.

- **Justification:** In (CITE), it is written, "[The unity between urea concentrations in the blood and the GIT] indicates that the rate of small-intestine bacterial urea metabolism is maximal at normal serum urea concentrations and further increases in plasma urea (eg, in renal failure) would not be expected to increase the urea metabolic rate. For example, Helicobacter pylori urease has a Km of 0.48 mM, sixfold lower than normal plasma urea (3.2 mM). If the rates of gastric and small-intestine bacterial urea metabolism were saturated at normal plasma-urea concentrations, it would also explain the "surprising" result that administering urea by mouth did not increase plasma-NH3 levels in cirrhotic liver patients." Therefore, it is appropriate to assume that the rate of urea breakdown by enteric bacteria under basal conditions is the maximum rate of breakdown.

The model of ammonia homeostasis developed by Levitt et al (CITE) found that the rate of urea breakdown by enteric bacteria under basal conditions is approximately $0.001\ \frac{mmols}{s}$ for a 70 kg human. From the above assumptions, the model uses this rate as the maximum rate of breakdown as well as a K_m value of 0.48 mM from the same paper to describe the rate of urea breakdown by enteric bacteria in the GIT:

$$\boxed{V_{Hydrolysis} = \frac{0.001 * U_{GIT}}{0.48 + U_{GIT}}} \tag{11}$$

where

$$[V] = \frac{mmols}{s}$$

7 Urea Breakdown by a Urease Treatment

7.1 Assumptions

- **Assumption:** The urease treatment will not be affected by the hostile conditions of the stomach, and its activity can be modeled as if it were function at 35 degrees Celsius and a pH of 7.
- **Justification:** The polymerization of urease will protect it from the hostile conditions of the stomach. Moreover, M-M constants were found only for a temperature of 35 degrees Celsius and the optimal pH for urease is 7 (CITE).
- **Assumption:** Sigma-Aldrich calculates the activity of its urease in saturated substrate conditions.
- **Justification:** For each batch of urease, Sigma-Aldrich calculates its activity at pH 7.0, 25 °Celsius, and a urea concentration of 455 mM (CITE). The K_m values for jack bean urease are on the order of 3 mM, far less than the concentration of urea at which Sigma-Aldrich calculated its urease's

activity. Therefore, it is reasonable to assume that the activity of urease Sigma-Aldrich reports for its urease products is the fast rate of urea breakdown.

Urea breakdown by urease behaves according to M-M kinetics. We will be using urease derived from jack beans and bought from Sigma-Aldrich. For each urease product, Sigma-Aldrich reports the "density" of the urease in $\frac{Units}{gram}$, where a unit "[w]ill liberate 1.0 umole of NH3 from urea per min at pH 7.0 at 25 °C" (CITE). To correct for the temperature difference, since Sigma-Aldrich calculated urease activity at 25 °cC but the body is at 35 °C, a corrective factor was calculated. The ratio of the maximum rates of jack bean urease activity at 35 °C versus 25 °C was 1.426 according to (CITE) and 1.419 according to (CITE). Thus, a corrective factor of 1.42 was used to account for the activity difference

Moreover, the K_m value for jack bean urease did not significantly change at varying temperatures according to (CITE), and was calculated to be 2.62 mM. According to (CITE), the K_m 4.6 mM at 35 °C. To account for the different K_m values reported, an average of 3.6 mM was used.

Urease activity is now able to be modeled as a function of its mass, in grams, and its "density", in Units/gram, as defined previously. The maximum rate of urea breakdown is calculated as

$$V_{max}\left(Mass, Density\right) = 1.42 * Mass * Density * \frac{10^3 mmols}{10^6 umols} * \frac{1\,min}{60\,s} * \frac{1\,mol\,NH_3}{2\,mols\,urea} \tag{12}$$

Inserting this maximum rate into an M-M equation, we get:

$$\boxed{V_{Urease} = \frac{V_{max} * U_{GIT}}{3.6 + U_{GIT}}} \tag{13}$$

where

$$[V] = \frac{mmols\,urea}{s}$$

(CITE) used Type IX jack bean urease and at 35 °C calculated a maximum rate of 0.002192 $\frac{mmols\,NH_3}{s}$ for 0.001 g urease. Type IX jack bean urease has a "density" 50,000-100,000 $\frac{Units}{gram}$. Assuming a density of 90,000 $\frac{Units}{gram}$, 0.001 grams urease, and 500 mM urea to simulate saturated conditions, the above equations produced a rate of 0.002120 $\frac{mmols\,NH_3}{s}$, very close to the rate found in (CITE), thus validating this modeling of the urease treatment.

8 Ammonia Production in the GIT

- **Assumption:** Ammonia production from the metabolism and absorption of glutamine by enterocytes is constant regardless of urea and ammonia concentration in the GIT and the blood.

- **Justification:** Glutamine metabolism and absorption are independent of ammonia concentration in the GIT and thus the amount of ammonia produced as a result is constant.

The rate of ammonia production is twice the rate of urea hydrolysis by enteric bacteria and the urease treatment because the hydrolysis of a urea molecule results in two molecules of ammonia. The Therefore,

$$\boxed{V_{Ammonia\ Production} = 2 * (V_{Hydrolysis} + V_{Urease}) + V_{Glutamine}} \tag{14}$$

where

$$V_{Glutamine} = 0.00138\ \frac{mmolsNH_3}{sec} \tag{15}$$

The rate of ammonia production from glutamine metabolism and absorption was retrieved from (CITE).

9 Ammonia Uptake from the GIT and Conversion to Urea

9.1 Assumptions

- **Assumption:** The fraction of ammonia that is absorbed into the portal vein from the small intestine is the same for all concentrations of ammonia in the GIT.
- **Justification:** (CITE) calculated that only 87 mmols of ammonia produced in the GIT enter the portal vein out of the 294 mmols produced per day under basal conditions. When more ammonia is produced than under basal conditions, due to the urease treatment, the same fraction is able to be absorbed before it is used by bacterial processes. Moreover, some bacteria proliferate under high concentrations of ammonia and some prefer it as a nitrogen source compared to other sources (CITE).
- **Assumption:** Ammonia production from the metabolism and absorption of glutamine by enterocytes is constant regardless of urea and ammonia concentration in the GIT and the blood.
- **Justification:** Glutamine metabolism and absorption are independent of ammonia concentration in the GIT and thus the amount of ammonia produced as a result is constant.
- **Assumption:** Ammonia that is absorbed into the portal vein is immediately either converted to urea by the liver or enters the systemic circulation.

- **Justification:** The first-pass system directs blood, with absorbed products such as ammonia, directly from the GIT to the liver. Therefore, it is appropriate to ignore the time it takes to go from the GIT to the liver because the portal blood will reach only the liver and nowhere else.

- **Assumption:** The rate of urea production discussed in section 4 takes into account the conversion of ammonia, from both the portal vein and systemic circulation, under basal conditions. The ammonia produced by the urease treatment adds onto this rate of urea production.

- **Justification:** The basal urea production rate already takes into account the conversion of ammonia into urea, as well as the production of urea from the metabolism of amino acids.

According to (CITE), of the 294 mmols of ammonia produced in the GIT per day, only 87, or about 29.6% enter the portal vein. The rest are presumed to be used by bacterial processes. The ammonia that enters the portal vein is delivered directly to the liver. Of the ammonia that passes through the liver, 70% is converted into urea and 30% enters the systemic circulation (CITE) - this filtration principle applies to both ammonia in the portal blood as well as in the systemic circulation.

The flow rate of systemic blood through the liver is 1.5 $\frac{L}{min}$. Applying the 85-15 conversion principle and this flow rate, we can calculate the rate of urea production from ammonia produced by the urease treatment and the rate of ammonia delivery and filtration to the systemic circulation:

$$\boxed{V_{Urea\,from\,Urease\,Ammonia} = 0.296 * 0.85 * \frac{1mol\,urea}{2mols\,NH_3} * 2 * V_{Urease}} \tag{16}$$

$$\boxed{V_{Systemic\,Ammonia\,from\,GIT} = 0.296 * 0.15 * \frac{1mol\,urea}{2mols\,NH_3} * V_{Ammonia\,Production}} \tag{17}$$

$$\boxed{V_{Systemic\,Ammonia\,Filtration} = 0.85 * \frac{1.5\,L}{60\,sec} * A_{blood}} \tag{18}$$

where

$$[V] = \frac{mmols}{s}$$

10 Ammonia Production and Uptake from Systemic Circulation

- **Assumption:** The rate of ammonia production by the kidneys is constant, regardless of GFR.

- **Justification:** The kidneys produce ammonia at a rate of 0.00025 $\frac{mmols}{s}$ under basal conditions directly to the systemic circulation, according to CITE. This rate decreases as a result of CKD, but is not well-quantified(?). Moreover, keeping this rate constant will simulate "worst-case" conditions because the kidneys would be delivering more ammonia to the blood than in reality even while the urease treatment is producing ammonia which will reach the systemic circulation as well.

- **Assumption:** Muscle uptake of ammonia is a constant rate

- **Justification:** Muscle uptake of ammonia increases as blood ammonia increases (CITE), yet this is not well-quantified. Similar to the assuming the rate of kidney ammonia production will simulate the "worst-case" scenario, a low rate of muscle uptake of ammonia will also simulate the "worst-case" scenario because less ammonia will be removed from the blood than in reality.

The net rate of ammonia production, directly into the systemic circulation, is simply the sum of kidney ammonia production and muscle ammonia uptake. According to (CITE), the rate of production and uptake are

$$V_{Kidney\,Ammonia\,Production} = 0.00025\frac{mmol}{s} \tag{19}$$

$$V_{Muscle\,Ammonia\,Uptake} = 0.0001\frac{mmol}{s} \tag{20}$$

Thus,

$$\boxed{V_{Systemic\,Ammonia\,Production} = V_{Kidney\,Ammonia\,Production} - V_{Muscle\,Ammonia\,Uptake} = 0.00015} \tag{21}$$

11 The Final Equations

11.1 Assumptions

- **Assumption:** The volume of blood in the body and fluid in the GIT is constant.

- **Justification:** The volume of blood in the body stays relatively constant throughout the day (?). The volume of fluid in the GIT varies with eating. However, one only eats, on average, 3 times per day and is fasted for the majority of the day, including between meals and during sleep. Moreover, this model is interested in longer term effects of a treatment and short, periodic changes in GIT fluid volume will not have a large effect on longer term effects of the treatment. Finally, BUN and ammonia tests are done when fasted, so assuming a constant, fasted volume of GIT fluid allows us to corroborate this model with real-life data.

The volume of the blood is about 5 L and the volume of the GIT fluid is 0.163 L fasted (CITE). To synthesize the above sections, the differential equations governing changes in urea concentrations in the blood and the stomach, and changes in ammonia concentration in the blood, are as follows:

$$\boxed{\frac{dU_{Blood}}{dt} = -V_{Blood \to GIT} + V_{GIT \to Blood} + V_{U\ prod.} + V_{U\ from\ Urease\ A} - V_{U\ excr.}} \tag{22}$$

$$\boxed{\frac{dU_{Stomach}}{dt} = V_{Blood \to GIT} - V_{GIT \to Blood} - V_{Hydrolysis} - V_{Urease}} \tag{23}$$

$$\boxed{\frac{dA_{Blood}}{dt} = V_{Sys.\ A\ from\ GIT} - V_{Sys.\ A\ Filtration} + V_{Sys.\ A\ Production}} \tag{24}$$