Tatiana Moyer MAT 598
Due 5/5/08 Final Project

MODELING MALARIA PATHOGENESIS

Biological Background and Motivating Questions:

Malaria, though widespread, is a disease that is still not widely understood. It is known, however, that the strain of malaria *Plasmodium falciparum* is more deadly and debilitating than the strain *Plasmodium vivax*. Many biologists and even mathematicians have tried to explain this by arguing that anemia and sequestration in *falciparum* account for this difference, but their hypotheses have proven faulty. Therefore our team set out to develop a model that would be more accurate to the biology of malaria, and we used a paper by Clark at al. as our basis which details the following parasitology of the disease.

The disease malaria begins when malarial glycosylphosphatidylinositols (GPIs) infect the body. The body responds by releasing proinflammatory cytokines such as tumor necrosis factor (TNF). This inflammatory response also generates oxygen-derived free radicals, which help to clear the parasites that are infecting the red blood cells. TNF also activates inducible nitric oxide synthase (iNOS), which generates nitric oxide (NO). NO helps to quell the inflammation so the inflammation doesn't get out of control. In this way, NO helps to combat malaria. However, NO also helps malaria because NO produces peroxynitrite, which nicks DNA. Cells then use poly (ADP-Ribose) polymerase family member 1 (PARP-1) to repair the damage. To fix the DNA, PARP-1 uses NAD^+ in such a way that in order for the body to replace the NAD^+, the body has to undergo ATP hydrolysis. When NO is great enough, PARP-1 depletes NAD^+ so much that the membrane-bound NA+/K+ APTase pump fails to operate properly. This failure

causes the cells to swell greatly and ultimately to get cleared by the spleen. This anemia serves to further deplete the cells of ATP. The ionic problems that occur due to the failure of the ATPase pump also erode muscle contractility so much that CO_2 can not be blown out of the cells. This further acidifies the blood, hurting its oxygen-carrying capabilities even more, and only further increasing anemia. In this way, the downward spiral of the disease continues.

Clark et al.'s model also includes many more details about malaria, which is very interesting to study because of this issue that the elements in the body that fight the disease also contribute to the infection. In fact, it is often the immune response and not the parasite that actually kills the infected person. This implies that sometimes the mechanisms of the disease should be ultimately helpful and sometimes they should be ultimately harmful to the body. Our group decided to focus on one aspect of Clark et al.'s model, the nitric oxide loop, because the biology detailed by Clark et al. is very complicated and the nitric oxide loop is a prime example of the both the positive and negative effects of the body's reaction to malaria. We want to study the instances in which the mechanisms in Clark et al.'s paper, specifically the nitric oxide loop, lead to pathology and the instances in which they lead to lead to clearance of the parasite. In other words, we want to see which will dominate: the therapeutic effects of the inflammation or the pathogenic effects of the inflammation. We can examine this dichotomy under the conditions of both *vivax* and *falciparum*, too, to look for differences in mortality. I would also like to point out that even though *falciparum* is more deadly, *vivax* still has devastating consequences because, aside from the fever being very painful,

it causes infected people to miss work and therefore to lose money. Whole communities suffer because people infected by *vivax* cannot do any work.

As a group project, we are still very much in the preliminary stages, but I still have many interesting results to share in this paper. We have not been able to answer our preliminary questions yet but we have the proper motivations guiding us. Eventually we hope to better define the boundary between these therapeutic and pathogenic conditions and see if we can even make predictions based on our model.

The Model

Our model at this point focuses on *Plasmodium falciparum*. We modified a model from Anderson, May, and Gupta (1989) which was also used by Iggidr et al. (2006). Our model is as follows:

$$x' = \Lambda - \mu_x x - Bxm - [\alpha j^s/(\gamma^s + j^s)]x$$

$$y' = Bxm - \mu_y y - [\alpha j^s/(\gamma^s + j^s)]y$$

$$m' = r\,\mu_y y - \mu_m m - Bxm$$

$$f' = ae^{-jk}y(t-\tau) - \mu_f f$$

$$j' = bf - \mu_j j$$

Variables are as follows:

VARIABLE	DESCRIPTION	UNITS
x	concentration of healthy red blood cells	cells / μL
y	concentration of infected red blood cells	cells / μL
m	concentration of free merozoites	cells / μL

f	concentration of TNF	pM
j	concentration of iNOS	pM

Parameters are as follows:

VARIABLE	DESCRIPTION	VALUE	UNITS
Λ	rate of erythropoesis	26560	cells / (µL*hour)
μ_x	death rate of healthy red blood cells	0.0083	1 / hour
β	contact/infection rate of the healthy red blood cells by merozoites	$8*10^{-6}$	µL / (cells*hour)
α	maximum percentage of RBCs killed via spleen due to NO-induced ATPase damage	0.8	1 / hour
s	speed at which the percentage of RBCs removed by the spleen reaches 100%	2	unitless
γ	threshold for dramatic NO-induced ATPase damage	1	pM
μ_y	death rate of infected red blood cells	0.025	1 / hour
r	average number of free merozoites released per bursting infected red blood cell	24	unitless
μ_m	death rate of free merozoites	60	1 / hours
a	default rate of TNF upregulation	10	(µL*pM) / (cells*hour)
k	rate of TNF downregulation	0.1	1 / pM

	due to feedback of NO		
τ	time delay of TNF effect	2	hour
μ_f	rate of TNF degradation	3.47	1 / hour
b	upregulation rate of iNOS due to TNF	1	1 / hour
μ_j	rate of iNOS degradation	.347	1 / hour

The properties of nonnegativity and boundedness have been proven for all variables (see Appendix). We have also proven that the disease-free equilibrium is locally asymptotically stable when the reproductive rate R_0 is greater than one (see Appendix).

Our group decided to remove the variable we originally had for NO by using the biological assumption that NO has a very short life and we believe the concentration of NO is directly related to the concentration of iNOS, which generates NO. Thus we assume that a variable for NO will have the same dynamics as our variable for iNOS, and the values for NO will be some scalar multiple of our values for iNOS. We don't believe this change will hurt a main purpose of our model, which is to investigate specifically both the helpful and the harmful effects of NO. Another biological assumption our model takes is that the iNOS/NO loop is more important than the HO-1/CO loop that Clark also mentions. We believe we can assume this because the HO-1/CO loop mimics the iNOS/NO loop, and the iNOS/NO loop has the interesting mechanism of specifically being both helpful and harmful to the infected person.

Analysis of the Model

We've obtained many results and graphs from our model. Figure 1 is the graph of our model for our default parameter values.

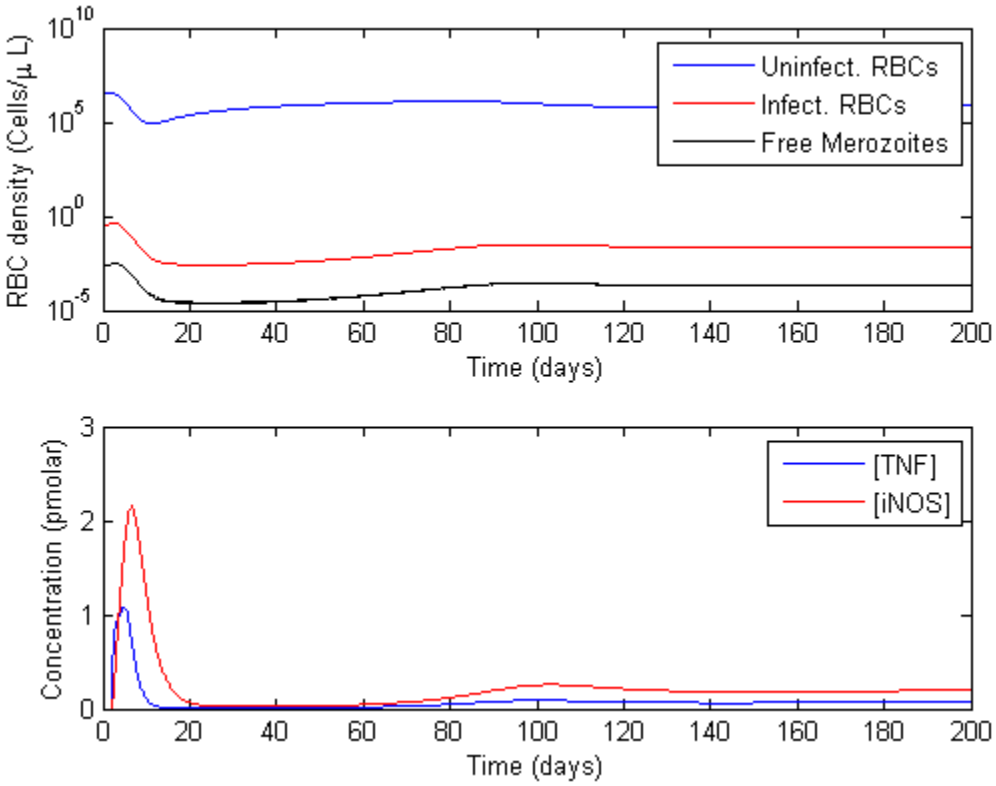

Figure 1: Model for Default Parameter Values

Note how in Figure 1 the variables appear to approach a stable value. We believe this is because the beta value, the contact/infection rate, and the tau value, the time delay, are small enough that the body can fight off the infection.

Figure 2 is the graph with all default values except beta increased to $8*10^{-4}$:

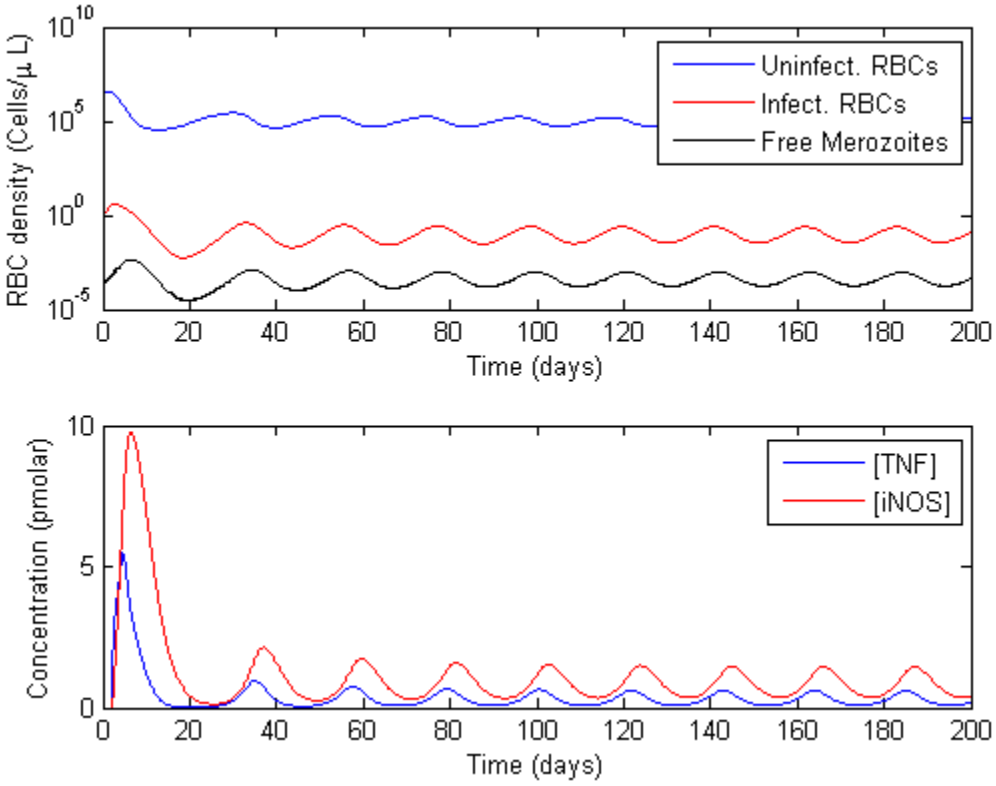

Figure 2: Model for Default Parameter Values Except $\beta=8*10^{-4}$

Note how the variables no longer approach a stable value. We believe the oscillations are indicative of a higher infection rate of RBC's so the body cannot fight off the infection and no longer approaches a chronic steady state. The oscillations are well known (as *falciparum* and *vivax* are tertian periodic and recur about every two days—our time scale may be off for now because of the magnitude of the differences in our parameters, which will be discussed later) in field research and are supported in the reviewed literature.

However, we believe that the oscillations that occur by "ramping up" beta are actually cause by the time delay tau. Remember that our default value for tau is 2. It appears from working with the model on MATLAB that the variables never oscillate unless the

tau value is greater than zero; for example, see the following graph with the beta value at $8*10^{-4}$ (the value for which oscillations occurred with otherwise default values) and the tau value at 0.1 (because the lags in the program have to be positive):

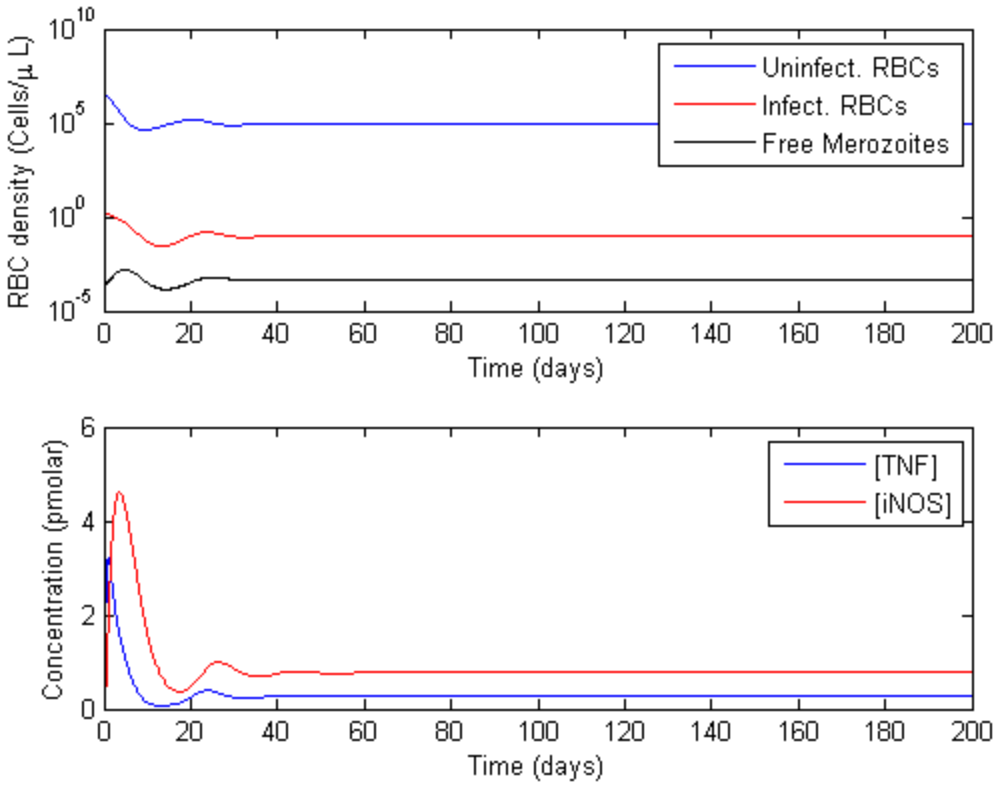

Figure 3: Model for Default Parameter Values Except β=8*10^{-4} and τ=0.1

Even though we believe tau causes the oscillations (because the time delay means the body cannot respond to the disease right away and therefore has a harder time clearing it —in other words, it can never quite "catch up" with clearing the disease), a positive value of tau alone will not cause oscillations. If oscillations are to occur, the infection rate beta value also has to be high enough; this is because oscillations mean the body is having a very difficult time fighting the infection, which only occurs if a time delay exists *and* the contact/infection rate (and thus the burden on the patient) is large enough. Note the

following graph that illustrates this principle in which the tau value is four but beta is still the smaller default value of $8*10^{-6}$:

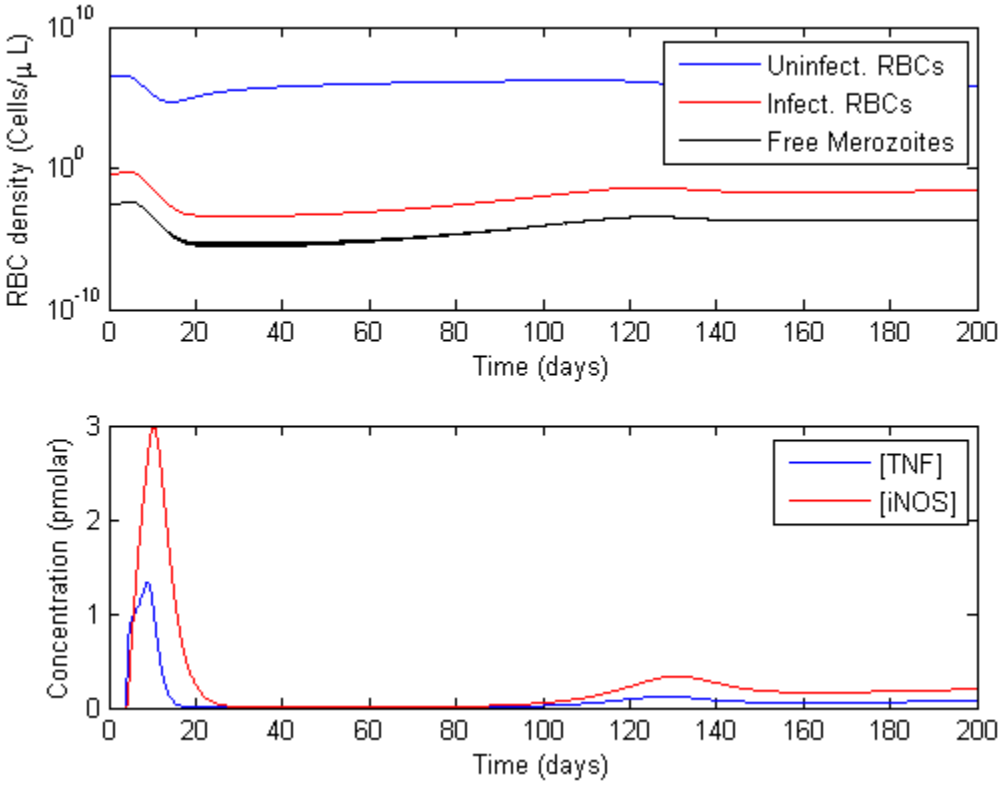

Figure 4: Model for Default Parameter Values Except τ=4

Oscillations do not occur in Figure 4 because even though tau is the fairly high value of 4, the beta value is not large enough to cause oscillations.

When beta is a high enough value to cause oscillations when the tau value is not 0 (say beta is $8*10^{-4}$), then oscillations *can* occur at low values of tau. For example, in Figure 5 for the beta value at $8*10^{-4}$, oscillations first start to appear at the tau value of 1.75:

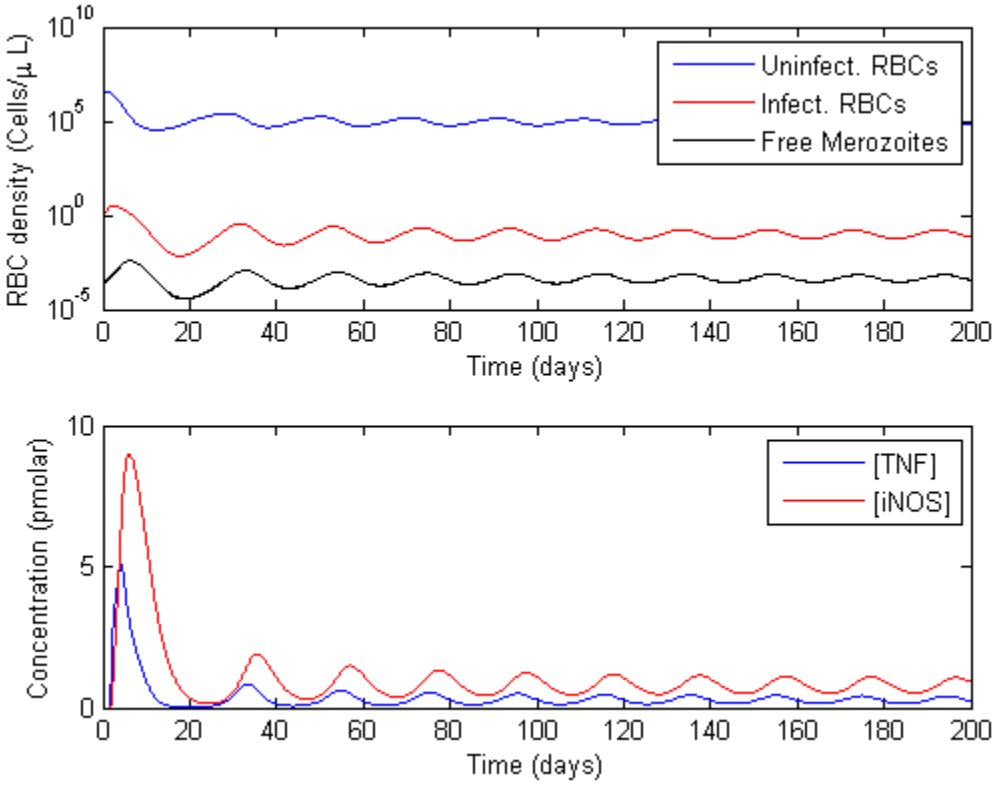

Figure 5: Model for Default Parameter Values Except $\beta=8*10^{-4}$ and $\tau=1.75$

We've also produced bifurcation diagrams for all of the variables versus beta, which is obviously a very important parameter since it gives the contact/infection rate.

Figure 6 gives the bifurcation diagram for beta versus x.

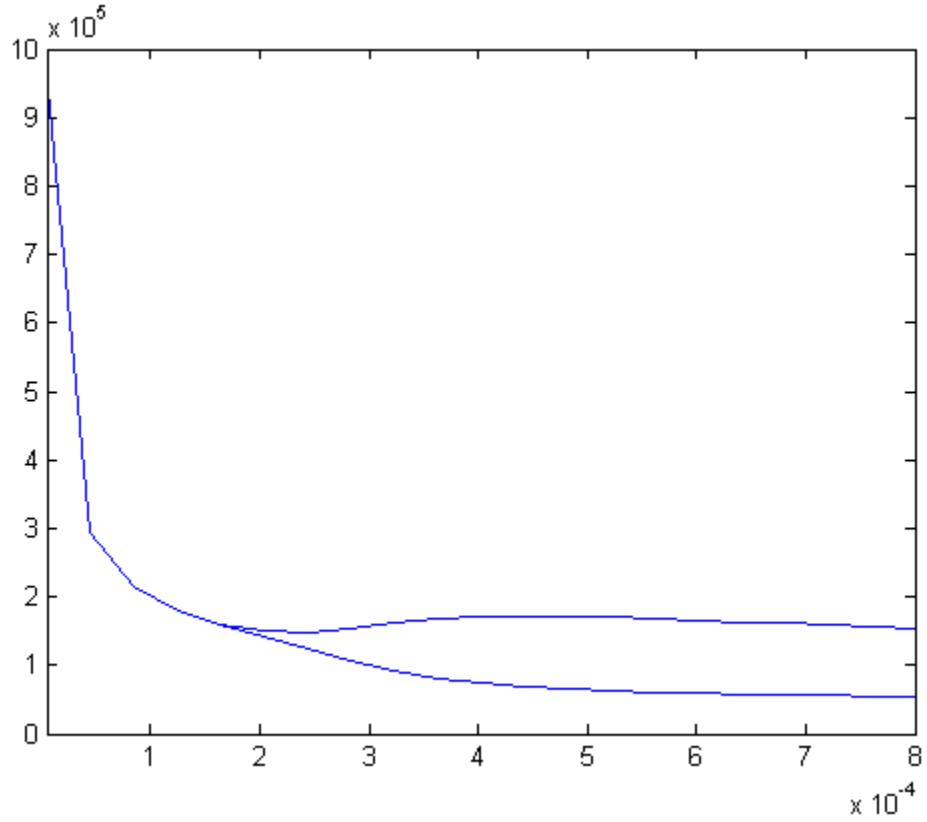

Figure 6: Bifurcation Diagram of β vs. x

Note that x bifurcates when the value of beta is roughly around $2*10^{-4}$. Then the period two oscillations observed in the previous diagrams occur, in which x oscillates between the values of about $5*10^{4}$ and $1.5*10^{5}$ at the beta value of $8*10^{-4}$.

As expected, the other bifurcation diagrams also bifurcate when the value of beta is roughly around $2*10^{-4}$. Figure 7 gives the bifurcation diagram for beta versus y.

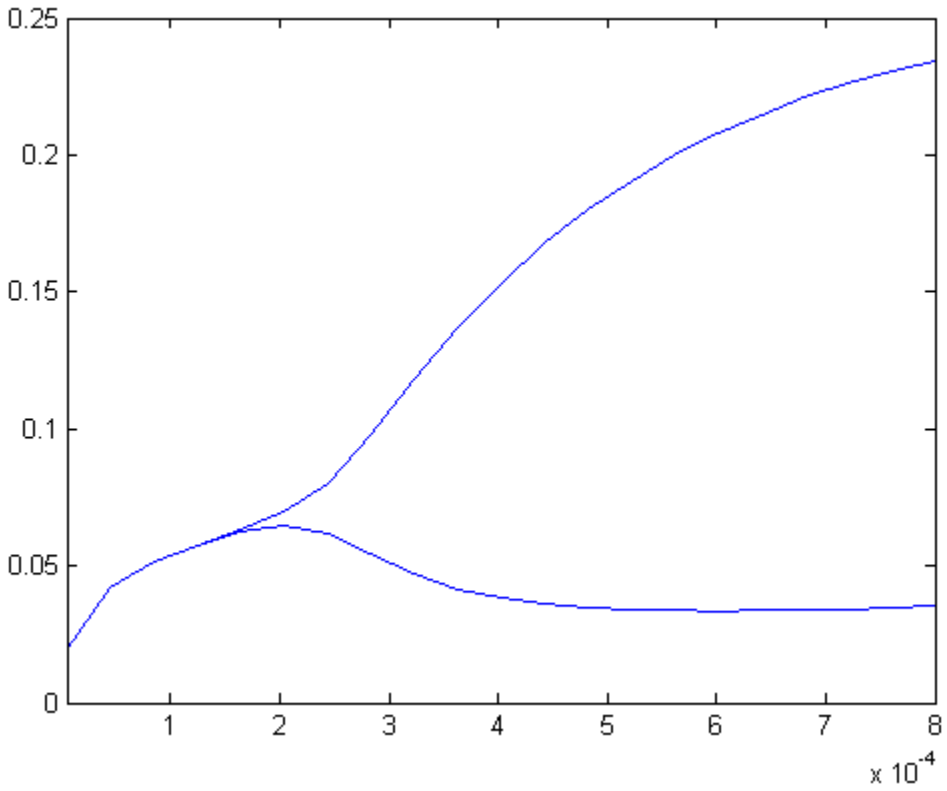

Figure 7: Bifurcation Diagram of β vs. y

The period two oscillations observed in the previous diagrams occur, in which the y value oscillates between about 0.04 and 0.23 at the beta value of $8*10^{-4}$. Figure 8 gives the bifurcation diagram for beta versus m.

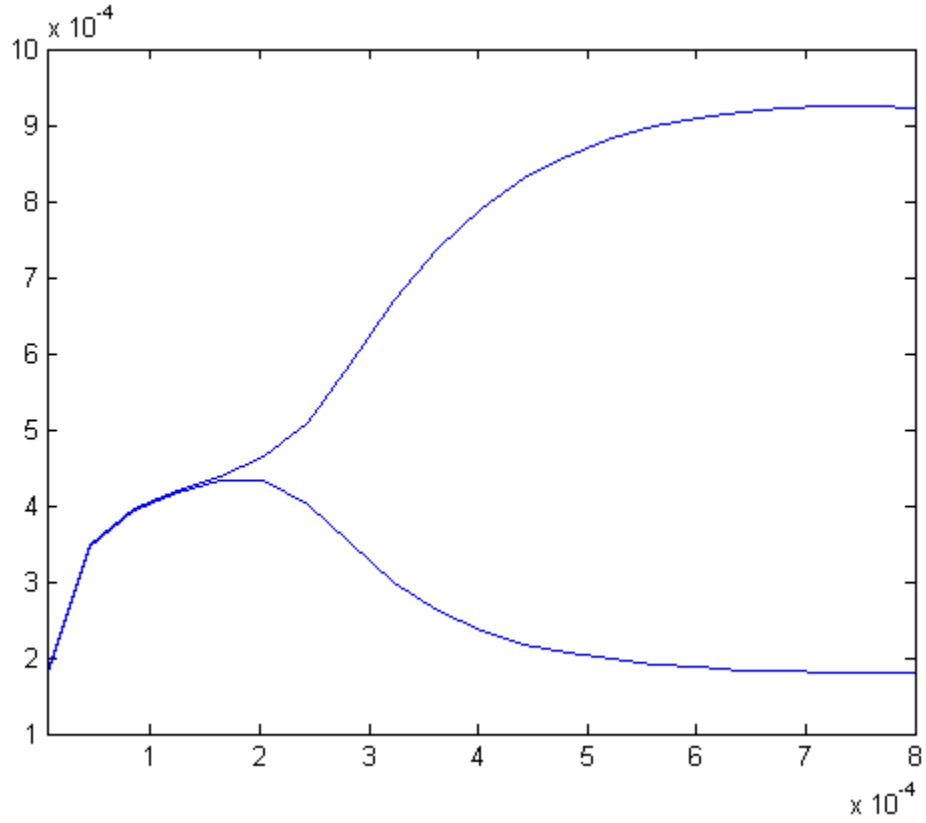

Figure 8: Bifurcation Diagram for β vs. m

The period two oscillations observed in the previous diagrams occur, in which the m value oscillates between about $1.9*10^{-4}$ and $9.2*10^{-4}$ at the beta value of $8*10^{-4}$. Figure 9 gives the bifurcation diagram for beta versus f.

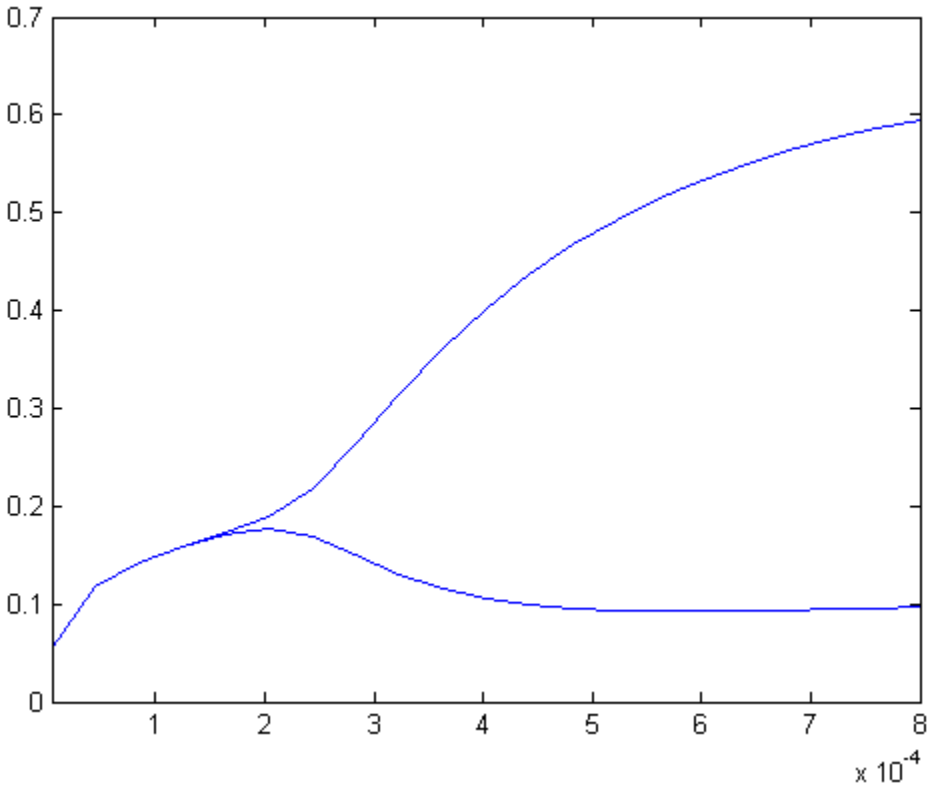

Figure 9: Bifurcation Diagram for β vs. f

The period two oscillations observed in the previous diagrams occur, in which the f value oscillates between about 0.1 and 0.6 at the beta value of $8*10^{-4}$. Figure 10 gives the bifurcation diagram for beta versus j.

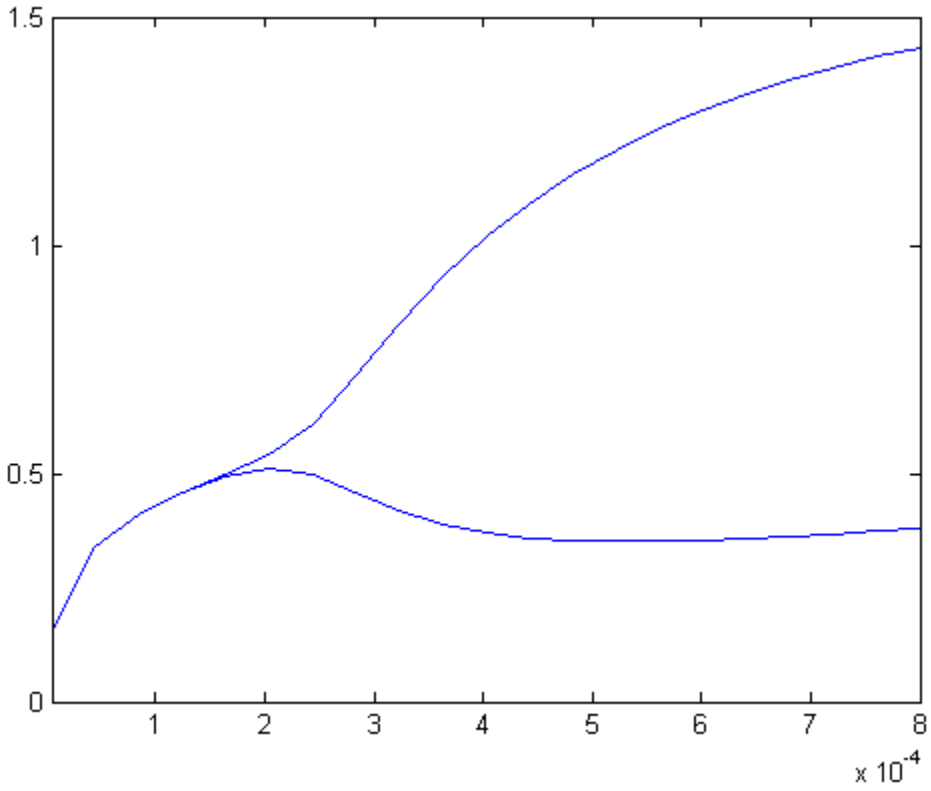

Figure 10: Bifurcation Diagram for β vs. j

The period two oscillations observed in the previous diagrams occur, in which the j value oscillates between about 0.4 and 1.4 at the beta value of $8*10^{-4}$.

Implications of our Findings and Future Work

One of the implications of our bifurcation diagrams is that the disease starts getting more out of control for the patient at a beta value of about $2*10^{-4}$, because this is when the oscillations first start to occur. These oscillations are confirmed in Figure 11.

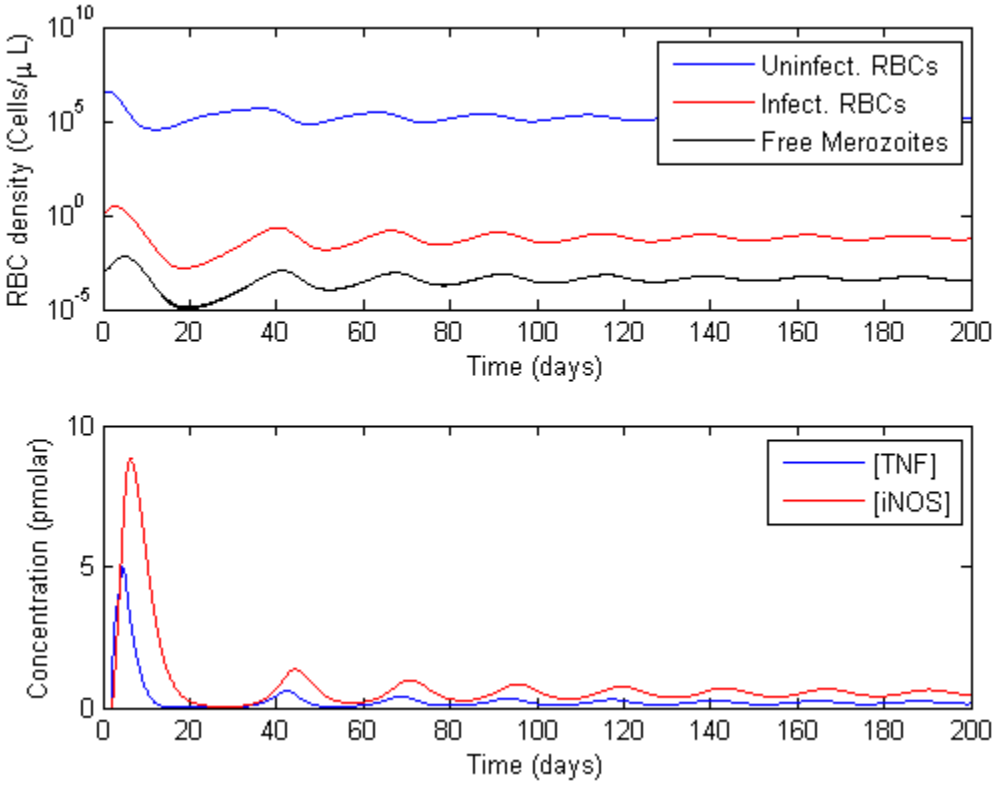

Figure 11: Model for Default Values Except β=2*10^{-4}.

Note that the iNOS level, and therefore the NO level, is almost quadrupled from the default model in Figure 1 to the model in Figure 11. The parasite load is also greater on average for the patient to handle, so the patient might not survive in the case of Figure 11. Thus, if the contact/infection rate given by beta could somehow be lowered in patients, they would have a better chance of survival.

Another implication of our diagrams is that the Hill function representation of anemia, while at first seemingly detrimental, actually helps to clear the parasite and is not entirely pathological. Thus sometimes the body is effective at clearing the malaria parasite. However, if the parasite load reaches a certain maximum level or if it remains at a high period for a long enough time, the patient could die. In the future we can graph

the variable (x+y) which is the total count of RBCs and look to see when this value becomes less than 40% because this is when a patient would normally die. We would like to examine when mortality occurs in the model to see more in depth how this immunopathology is itself therapeutic.

This is not our research level model that we will use when we are working to achieve publication. As has been pointed out to us, the difference between magnitudes in our parameters is too high, so we must further confirm the values of our parameters. We also plan on making a quasi-steady state assumption to fix this problem.

We also plan on adding a –U(f)y term to the y' equation and possibly a +U(f)x term to the x' equation to illustrate the therapeutic effect the inflammation response has against malaria; we just aren't sure yet whether or not this therapeutic effect fixes the infected red blood cells (making them healthy red blood cells again) or whether it just destroys infected red blood cells.

The "double-edged sword" qualities of malaria pathogenesis make it very difficult to fight by vaccination. As Clark et al. point out, getting rid of malaria GPIs will decrease the burden of the parasite on the patient, but this will also reduce the patient's response of fighting the parasite. We hope that when we work further on our model, if we highlight the boundary between the therapeutic and pathogenic effects of the disease mechanisms, specifically NO, and if we see how tweaking parameters can lower the parasite load, we can shed some light on this issue.

Bibliography

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