

# The Dynamics of Bacterial Infection, Innate Immune Response, and Antibiotic Treatment

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December 1, 2006

## Abstract

We develop a simple mathematical model of a bacterial colonization of host tissue which takes account of nutrient availability and innate immune response. The model features an infection-free state which is locally but not globally attracting implying that a super-threshold bacterial inoculum is required for successful colonization and tissue infection. A subset  $B$  of the domain of attraction of the disease-free state is explicitly identified. The dynamics of antibiotic treatment of the infection is also considered. Successful treatment results if the antibiotic dosing regime drives the state of the system into  $B$ .

Keywords: pharmacokinetics, pharmacodynamics, antibiotic treatment, infection.

## 1 Introduction

Mathematical modeling of the effects of drug treatment has long been used side-by-side with experimental studies [2, 5, 7, 8, 21, 24, 33]. However, most mathematical models of the effects of antimicrobial agents on bacterial populations assume that bacteria grow at an exponential rate in the absence of the antimicrobial agent [22, 13] or assume logistic growth of bacteria in the absence of treatment [2, 21, 14]. This seems surprising given that in vitro experiments often use the chemostat [9, 22] and many researchers point out that levels of certain key nutrients are critical to successful colonization of tissue by pathogens. As noted in Brock [3], since the initial pathogen inoculum is usually very small, its survival and ability to colonize depends critically on the availability of nutrients. Cozens et al [9] note that the restricted availability of iron and other nutrients appear to be typical of infection states. It is noted in [7, 9] that bacteria multiply more slowly in an experimental animals than in vitro suggesting

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\*Supported by NSF Grant DMS 0414270

nutrient limitation in vivo. Therefore, it seems natural to us that the basic Monod model of microbial growth under nutrient limitation should be at the core of models that include the population dynamics of the pathogen. We introduce this idea in [15, 16].

Most modeling efforts including those site above leave out an immune response to bacterial invasion. An exception is the unpublished paper of Levin and Antia [14]. The effect of this exclusion is that, depending on parameters, the infection-free steady state is either globally attracting or there is an infection steady state that attracts all solutions except the infection-free state. However, it is widely observed that a critical determinant of successful colonization and infection is the size of the inoculum. Murray et al [18] note that fewer than 200 *Shigella* suffice for Shigella and  $O(10^8)$  of *Campylobacter* are required for GI tract infections. The notion of a minimal infecting dose is usually expressed as I.D.50, the quantity causing infection in 50% of a suitable series of animals or cells (cell cultures). This suggests that while the infection-free state may be locally stable, it may not be stable to a large inoculum. It therefore seems necessary to include an immune response in order to capture this “threshold dose” phenomena. We construct a simple model of colonization and infection based on the Monod model for bacterial growth on a limiting nutrient and including bacterial predation by phagocytes of the host immune system. Our model exhibits bi-stability when local nutrient levels exceed a threshold; the infection-free state is globally attracting for subthreshold nutrient levels. Consequently, our model suggests that one mode of treatment would be to lower nutrient levels at the infection site. However, identifying the limiting nutrient at an infection site and finding means to lower it is probably infeasible.

We consider treatment of infection by antibiotic dosing. Standard compartmental pharmacokinetic models are employed to model the delivery of antibiotic to the infection site. General time-dependent dosing is considered making the treatment model non-autonomous.

It is often found that fast growing bacteria are more susceptible to antibiotic treatment as compared to slow growing ones [5, 9, 17, 23, 25, 31, 32]. A slow growth rate due to the restricted availability of nutrients could be a major contributor toward insensitivity of bacteria to antibiotic. Therefore it is plausible that the pharmacodynamic function, which quantifies the dependence of drug effect on drug concentration, should depend on both the antimicrobial agent and limiting nutrient levels. Corpet et al [7] introduce pharmacodynamic functions which depend on both limiting nutrient and antimicrobial agent. Cogan [5] does as well in his considerations of persister cells. Roberts and Stewart [25] construct a mathematical model to explore the possibility that the observed antibiotic tolerance of biofilms is due in part to nutrient limitation reducing bacterial growth and hence killing rates.

We assume the pharmacodynamic function depends on both antibiotic and limiting nutrient, making minimal assumptions of its dependence on these quantities that are satisfied for nearly all examples found in the literature.

Our main results for the treatment model include explicitly identifying a minimal value of phagocyte density and a maximal value of bacterial density having the property that any treatment regime that drives phagocyte density above the minimal level and bacterial density below the maximal level may be immediately terminated at that time with the assurance that successful clearance of the infection ensues. We then show that dosing regimes that drive down bacterial density to sufficiently low levels will allow phagocyte levels to recover to super-threshold levels and thereby achieve successful treatment. Simulations explore various features of drug treatment including both successful and unsuccessful treatment regimes.

## 2 Model of Infection Process

We seek a minimal model of the infection process that takes account of the importance of nutrient availability to the success of bacterial colonization of a tissue and that contains a simplified nonspecific immune response. Let  $S$  represent nutrient concentration,  $v$  the density of phagocytes (cells/volume), and  $u$  the density of bacteria (cells/volume) at the infection site.

A standard handling time argument is used to derive the rate of bacteria killing by a phagocyte. Assume that in time  $T$ , a phagocyte processes  $n$  bacterial cells. If it spends its time searching for bacteria at rate  $s$  (volume/time) and requires  $b$  units of time to process a single bacteria, then

$$n = (T - nb)su$$

and therefore the rate of bacterial killing per phagocyte is

$$\frac{n}{T} = \frac{su}{1 + bsu}$$

The rate of killing of bacteria by phagocytes is then

$$\frac{du}{dt} = -\frac{su}{1 + bsu}v$$

If we assume that a phagocyte kills on average  $N$  bacterial cells before dying, then the rate of loss of neutrophils and bacteria are related

$$N\frac{dv}{dt} = \frac{du}{dt}$$

This leads to the equations

$$\begin{aligned} S' &= d_S(S^0 - S) - \gamma^{-1}f(S)u \\ v' &= d_v(v^0 - v) - h(u)v \\ u' &= f(S)u - d_uu - Nh(u)v \end{aligned} \tag{1}$$

where

$$h(u) = \frac{su}{N(1 + bsu)}.$$

Classical Monod growth kinetics

$$f(S) = \frac{mS}{a + S}$$

are used with  $\gamma$  denoting the growth yield. The removal rates  $d_S, d_v, d_u > 0$  describe the turnover of  $S, v, u$ .  $d_u$  may reflect removal by advection or possibly by the complement system, an immune system component made up of various proteins that act against invaders. The products  $d_S S^0$  and  $d_v v^0$  give the flux of nutrient and flux of phagocytes into the tissue environment.

**Remark 2.1** *Actually, phagocytes like the common neutrophil are actively recruited to the infection site via chemotaxis along a gradient of a chemical messenger emitted by the complement system and by macrophages. This could be crudely modeled by allowing the flux into the infection site to depend positively on bacterial density. For example, the flux term  $d_v v^0$  might be replaced by*

$$d_v v^0 + \frac{\mu u}{\chi + u}$$

where  $\mu, \chi > 0$ . This embellishment of the model would not affect our main conclusions.

Scaling variables

$$\bar{S} = S/a, \quad \bar{v} = v/v^0, \quad \bar{u} = u/a\gamma \quad (2)$$

and defining

$$\lambda = Nv^0/(a\gamma), \quad \bar{S}^0 = S^0/a$$

and

$$F(\bar{S}) = f(a\bar{S}), \quad H(\bar{u}) = h(a\gamma\bar{u})$$

we obtain the scaled equations (dropping the bars):

$$\begin{aligned} S' &= d_S(S^0 - S) - F(S)u \\ v' &= d_v(1 - v) - H(u)v \\ u' &= F(S)u - d_u u - \lambda H(u)v \end{aligned} \quad (3)$$

with

$$F(S) = \frac{mS}{1 + S}, \quad H(u) = \frac{qu}{r + u}$$

where  $q = 1/Nb$  and  $r = 1/b\gamma$ .

Solutions of (3) are uniformly ultimately bounded if  $d_u > 0$ . An explicit ultimate upper bound for  $u$  will prove useful.

**Proposition 2.1** *If  $d_u > 0$ , then*

$$\limsup_{t \rightarrow \infty} (S + u) \leq \frac{d_S S^0}{\min\{d_S, d_u\}}$$

and (3) has a compact global attractor.

**Proof:** Adding the first and last equation gives

$$S' + u' \leq d_S(S^0 - S) - d_u u \leq d_S S^0 - \min\{d_S, d_u\}(S + u).$$

Integration leads directly to the desired conclusion. ■

It is interesting that solutions can become unbounded if  $d_u = 0$ , and that bacterial growth is linear with time, not exponential as one may guess.

The Sterile or Infection-Free state is given by:

$$S = S^0, \quad v = 1, \quad u = 0 \quad (4)$$

Its stability is determined by the invasion eigenvalue

$$\Lambda = F(S^0) - d_u - \lambda H'(0) = F(S^0) - d_u - \frac{\lambda q}{r} \quad (5)$$

In a healthy host, one expects that  $\Lambda < 0$  so the infection-free state is at least locally stable-it can handle small doses of bacteria. However, it may not be globally stable; a large initial inoculum of bacteria may overwhelm the immune response leading to infection. Figure 1 confirms this expectation, depicting immune system suppression of a sub-threshold inoculum and depicting a super-threshold inoculum developing into an infection state.

Obtaining biologically reasonable parameter values for our model is a challenge. First, we must identify the limiting nutrient for bacteria and determine the Monod parameters  $m, a$  and the yield  $\gamma$ , the flux of that nutrient into the infection site  $d_S S^0$  and its removal rate. We have chosen rates appropriate for *E. coli* growing on glucose  $m = 1.35/hr$ ,  $a = 4 \times 10^{-7} gm/ml$ , and  $\gamma = 0.41 \times 10^{12} cells/gm$ . The nutrient removal rate is chosen as  $d_S = 0.1/hr$  implying an approximate 10 hour residence time for nutrient and  $S^0 = 4a$ . Parameter values for phagocytes are difficult to come by. We have tried to make correspondences with the unpublished manuscript of Levin and Antia [14]. They take phagocyte removal rate  $d_v = 0.05$  cells/hr and their maximum phagocytosis rate  $\gamma_M = 10^{-5}$  corresponds with our parameter  $s$ . Their maximum influx rate of phagocytes is  $V_M = 10^4$  cells/hr which we equate to  $d_v v^0$ . Phagocytes consist of macrophages and neutrophils. We do not have a supported value for  $N$  but if we take  $N = 5$ , then we get  $\lambda = N v^0 / a \gamma = 6$ . We do not find any value for the handling time  $b$  from the literature so we choose ten minutes or  $b = 1/6$  hour. The value of  $q = 1/Nb$  and  $r = 1/bsa\gamma$  should have ratio  $q/r = sa\gamma/N = .33$ . With our value of  $b$ , we have  $q = 1.2$  and  $r = 3.6$ . Finally, we take  $d_u = d_S = 0.1$ . We make no claim that these values are accurate. These values are used in Figure 1.

It is instructive to express the eigenvalue  $\Lambda$  in terms of the original unscaled parameters:

$$\Lambda = f(S^0) - d_u - s v^0.$$

It depends on the bacterial growth rate, which depends on nutrient availability  $S^0$  at the infection site, the bacterial removal rate  $d_u$  and on  $s(d_v v^0)/d_v$ . The last term involves the phagocyte search rate, flux of phagocytes into the infection site, and their removal rate. We may view  $1/(d_u + s v^0)$  as the mean bacterial life span from which the basic reproductive number for bacteria is

$$R_0 = \frac{f(S^0)}{d_u + s v^0}$$

A steady state at which  $u > 0$  will be called an infection steady state. Straight forward algebra leads to the following equations for such a state  $(S, v, u)$ :

$$\begin{aligned} u &= \frac{d_S(S^0 - S)}{F(S)} \\ v &= \frac{d_v}{d_v + H(u)} = \frac{d_v(r + u)}{d_v(r + u) + qu} \\ F(S) - d_u &= \frac{\lambda d_v q}{d_v(r + u) + qu} \end{aligned}$$

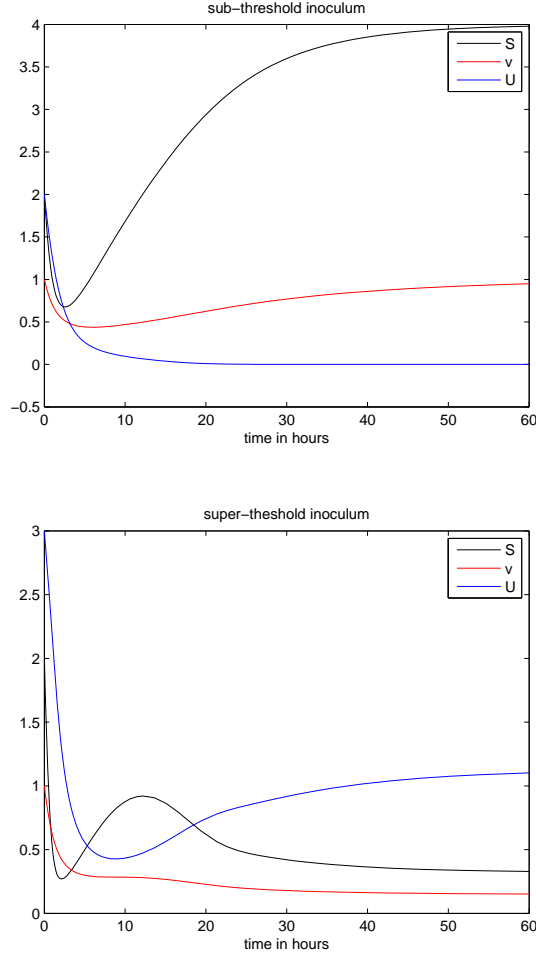


Figure 1:  $S(0) = S^0 = 2$ ,  $v(0) = v^0 = 1$ , top  $u(0) = 2$ , bottom  $u(0) = 3$

The first equation says  $0 < S < S^0$  and the third says  $S > F^{-1}(d_u)$  so we must have  $F(S^0) > d_u$ . Putting these two equations together, we arrive at a single equation for  $S \in (F^{-1}(d_u), S^0)$ :

$$d_S(S^0 - S)(F(S) - d_u) = \eta F(S) \left[ \frac{\lambda q}{r} + d_u - F(S) \right] \quad (6)$$

where

$$\eta = \frac{rd_v}{d_v + q}$$

The left side vanishes at the endpoints of  $(F^{-1}(d_u), S^0)$  and is positive in between; the right side is positive at  $F^{-1}(d_u)$  and it has the sign of  $-\Lambda$  at  $S^0$ . Multiplying the equation by  $(1 + S)^2$ , we see that it is a cubic in  $S$  with the cubic terms on the left.

We summarize our understanding of the dynamics of (3) below.

**Theorem 2.1** *The infection-free state is stable if  $\Lambda < 0$  and unstable if  $\Lambda > 0$ . If  $\Lambda > 0$ , then  $u$  is uniformly persistent and there exists exactly one infection steady state. If  $\Lambda < 0$  then at most two infection states exist.*

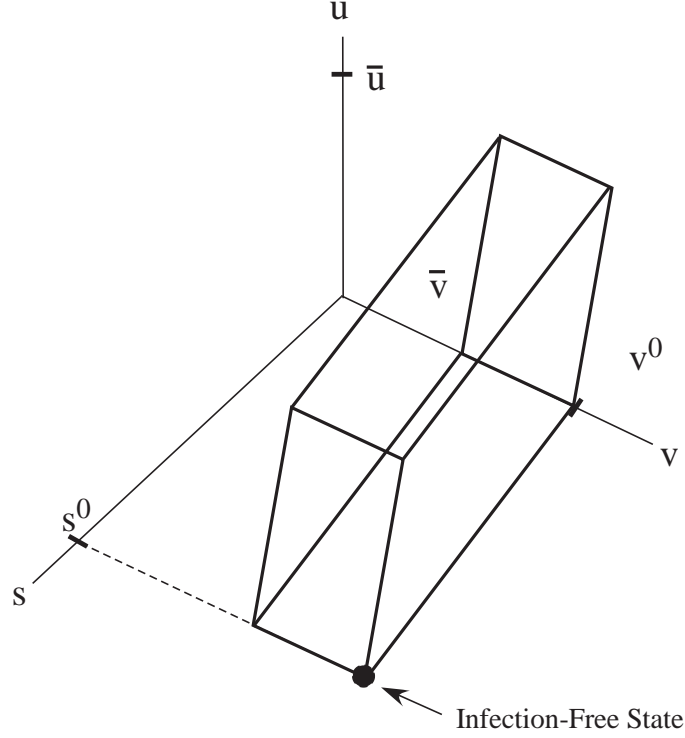


Figure 2: Box  $B$  is contained in the basin of attraction of  $(S^0, 1, 0)$ .

If  $F(S^0) - d_u - \lambda H'(M) \frac{d_v}{d_v + H(M)} < 0$  where  $M = \frac{d_S S^0}{\min\{d_S, d_u\}}$  then the disease-free state is globally attracting.

If  $F(S^0) > d_u$ ,  $\Lambda < 0$ , and

$$\bar{u} = \frac{d_v r}{d_v + q} \left[ \frac{-\Lambda}{F(S^0) - d_u} \right]$$

then  $\bar{u} > 0$ ,  $\bar{v} = \frac{d_v}{d_v + H(\bar{u})} > 0$ . Moreover, if

$$(S(0), v(0), u(0)) \in [0, S^0] \times [\bar{v}, 1] \times [0, \bar{u}] \equiv B \quad (7)$$

then  $(S(t), v(t), u(t)) \rightarrow (S^0, 1, 0)$  as  $t \rightarrow \infty$ .  $B$  is positively invariant.

**Proof:** The stability assertions are trivial and the persistence assertion follows from well-known results (see e.g. Thieme [29]) since (1) the dynamics on the invariant set  $u = 0$  are trivial, and (2) there exists a global compact attractor for the dynamics of (3). To see the latter, just add the first and last equation and use standard differential inequality arguments.

The existence of an infection state when  $\Lambda > 0$  follows from the intermediate value theorem and the fact that the right hand side of (6) is negative at  $S^0$  while the left hand side vanishes there and the left hand side vanishes at  $F^{-1}(d_u)$  while the right hand side is positive there. Uniqueness is a consequence of the fact that solutions of (6) are also solutions of a cubic polynomial as noted above. This polynomial has one negative root and one root larger than  $S^0$  so all three roots are accounted for.

If  $\Lambda < 0$ , then the right hand side of (6) is positive on  $(0, S^0]$ . If  $F(S^0) \leq d_u$  then the right hand side is non-positive so there can be no infection state. As in the previous paragraph, the cubic polynomial has a negative root so there can be at most two others.

It is easy to see that if  $S(t_0) \leq S^0$  then  $S(t) \leq S^0$  for all  $t \geq t_0$ . In fact, every solution  $(S(t), v(t), u(t))$  satisfies  $S(t) \leq S^0$  for some, and hence all, large  $t$ . Similarly, if  $v(t_0) \leq 1$ , then  $v(t) \leq 1$ ,  $t \geq t_0$  and every solution satisfies  $v(t) \leq 1$  for all large  $t$ . It suffices to consider only solutions which start (and remain) satisfying  $S(t) \leq S^0$ ,  $v(t) \leq 1$  and  $v(t) > 0$ .

If  $F(S^0) - d_u - \lambda H'(M) \frac{d_v}{d_v + H(M)} < 0$  then it is negative when we replace  $M$  by an  $N > M$ . By Proposition 2.1,  $u^\infty = \limsup_{t \rightarrow \infty} u(t) \leq M$  so there exists  $t_0 > 0$  such that  $u(t) < N$ ,  $t \geq t_0$ . We use the symbols  $w^\infty, w_\infty$  for the limit superior/limit inferior of a function  $w$  defined on  $t \geq 0$ .  $v$  satisfies  $v' \geq d_v - (d_v + H(M))v$ ,  $t \geq t_0$  so  $v_\infty \geq \frac{d_v}{d_v + H(M)}$  and we can choose  $t_0$  larger if necessary so that  $v(t) \geq \frac{d_v}{d_v + H(N)}$ ,  $t \geq t_0$ . The inequalities  $H(u)/u > H'(u) \geq H'(M) \geq H'(N)$  for  $u \in (0, M]$  imply that

$$u'/u = F(S) - d_u - \lambda \frac{H(u)}{u} v \leq F(S^0) - d_u - \lambda H'(N) \frac{d_v}{d_v + H(N)} < 0, \quad t \geq t_0.$$

Therefore,  $u(t) \rightarrow 0$ .

Suppose  $F(S^0) > d_u$ . The planar system

$$\begin{aligned} v' &= d_v(1 - v) - H(u)v \\ u' &= F(S^0)u - d_u u - \lambda H(u)v \end{aligned} \quad (8)$$

is competitive on  $\mathbb{R}_+^2$  since the off-diagonal entries of its jacobian are negative. It therefore generates a monotone system relative to the fourth quadrant order relation:

$$(v, u) \leq_K (V, U) \Leftrightarrow v \leq V, \quad u \geq U$$

(8) has the asymptotically stable equilibrium  $(1, 0)$ . Since  $F(S^0) > d_u$  there is another equilibrium  $(\bar{v}, \bar{u})$  and it satisfies  $(\bar{v}, \bar{u}) \leq_K (1, 0)$ . The Jacobian at  $(\bar{v}, \bar{u})$

$$\begin{pmatrix} -d_v/\bar{v} & -H'(\bar{u})\bar{v} \\ -\lambda H(\bar{u}) & F(S^0) - d_u - \lambda \bar{v} H'(\bar{u}) \end{pmatrix}$$

has negative determinant because  $\bar{u}$  satisfies

$$0 = F(S^0) - d_u - \lambda \bar{v} \frac{H(\bar{u})}{\bar{u}} < F(S^0) - d_u - \lambda \bar{v} H'(\bar{u}),$$

the last inequality due to concavity of  $H$ . Thus  $(\bar{v}, \bar{u})$  is a saddle point.

Every bounded solution of a competitive planar system converges to equilibrium (see e.g., [27]). Standard comparison arguments imply that every solution  $(v(t), u(t))$  of (8) with  $(\bar{v}, \bar{u}) \leq_K (v(0), u(0)) \leq_K (1, 0)$  and  $(\bar{v}, \bar{u}) \neq (v(0), u(0))$  satisfies  $(v(t), u(t)) \rightarrow (1, 0)$  as  $t \rightarrow \infty$ . Furthermore, the set  $D = \{(v, u) : (\bar{v}, \bar{u}) \leq_K (v, u) \leq_K (1, 0)\}$  is positively invariant for (8). Observe that  $B = [0, S^0] \times D$ .

A solution  $(S(t), v(t), u(t))$  of (3) satisfies

$$\begin{aligned} v' &= d_v(1 - v) - H(u)v \\ u' &\leq F(S^0)u - d_u u - \lambda H(u)v \end{aligned}$$

and by standard differential inequality arguments (see e.g., appendix B of [26]), we have

$$(v(t), u(t)) \geq_K (V(t), U(t)), t \geq 0$$

where  $(V(t), U(t))$  is the solution of (8) that agrees with  $(v(t), u(t))$  at  $t = 0$ . This means that

$$v(t) \geq V(t), u(t) \leq U(t), t \geq 0$$

If  $(S(0), v(0), u(0)) \in B$ , then  $(V(0), U(0)) = (v(0), u(0)) \in D$  so  $(V(t), U(t)) \in D, t \geq 0$  and  $\bar{v} \leq V(t)$  and  $U(t) \leq \bar{u}$ . As noted above,  $S(t) \leq S^0, v(t) \leq 1$  and  $u(t) \geq 0$  for  $t \geq 0$ . Therefore, we see that  $B$  is positively invariant for (3). If  $(v(0), u(0)) \neq (\bar{v}, \bar{u})$ , then  $(V(t), U(t)) \rightarrow (1, 0)$  implying that  $u(t) \rightarrow 0$  and  $v(t) \rightarrow 1$ . As  $B$  is positively invariant, we can apply this same argument so long as  $(v(t), u(t)) \neq (\bar{v}, \bar{u})$  for some  $t \geq 0$ . But  $(v(t), u(t)) = (\bar{v}, \bar{u})$  for all  $t \geq 0$  implies  $(S(t), v(t), u(t)) = (S^0, \bar{v}, \bar{u}), t \geq 0$ , a contradiction since the latter is not an equilibrium. We conclude that every solution starting in  $B$  converges to the infection-free state.  $\blacksquare$

We view that last assertion in the case that  $\Lambda < 0$  and  $F(S^0) > d_u$  to be the main result. We argued above that  $\Lambda < 0$  is the healthy host condition. The reverse inequality  $F(S^0) \leq d_u$  implies global stability of the infection-free state so the case  $F(S^0) > d_u$  is a necessary condition for the existence of an infection state. This last assertion provides an explicit subdomain of the basin of attraction of the infection-free state. As we regard the restrictions  $S(0) \leq S^0$  and  $v(0) \leq v^0 = 1$  to be natural ones, we may interpret this result as providing a minimal phagocyte level  $\bar{v}$  and a maximal bacterial level  $\bar{u}$  such that if at some time the phagocyte level exceeds this threshold while bacterial levels do not exceed the bacterial threshold then bacterial colonization fails. This will provide an important set of target conditions for treatment

Figure 3 shows the effect of varying the nutrient influx rate  $S^0$  on equilibrium amplitude. Except for  $S^0$ , the bifurcation parameter, other parameters are as described above. Clearly, the nutrient influx rate has an impact on infection dynamics. Indeed, it suggests that reducing nutrient at the infection site would be an effective treatment.

### 3 Antibiotic Treatment

Modeling drug treatment using the standard compartmental approach requires identifying the compartment (e.g., mouth, blood, intramuscular) into which the drug enters, the various intermediate compartments, and the actual site of infection. If drug enters the first compartment and the tissue is the final compartment, then the vector  $x = (x_1, x_2, \dots, x_n)^T$ , whose components are the amount of drug in the compartments, satisfies:

$$x' = Cx + r(t)e_1^T, x(0) \geq 0 \tag{9}$$

where we denote the standard basis vectors for  $\mathbb{R}^n$  by  $e_i$  and  $C$  satisfies  $c_{ij} \geq 0, i \neq j$  and has non-positive column sums.  $c_{ij}$  is the rate of flow, per unit mass, from compartment  $j$  to compartment  $i$  for  $i \neq j$ . We assume that  $C$  is a stable matrix, which is the case if it is nonsingular or if it is irreducible. We also assume that there is a directed path

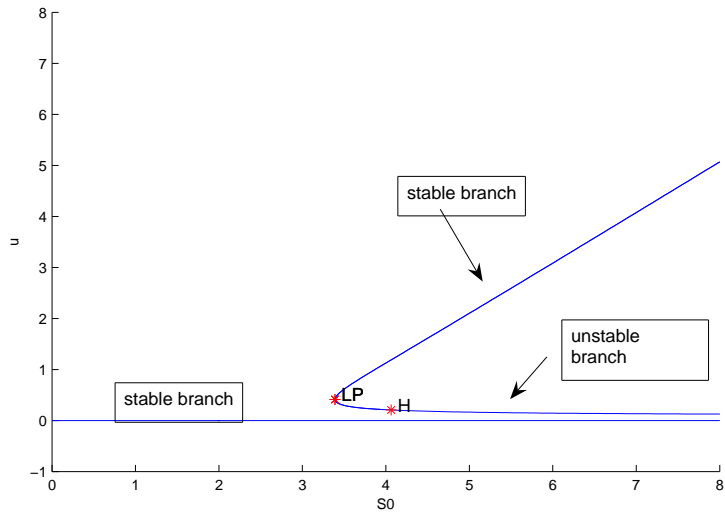


Figure 3: Bifurcation diagram: amplitude  $u$  of infection equilibrium versus  $S^0$ .

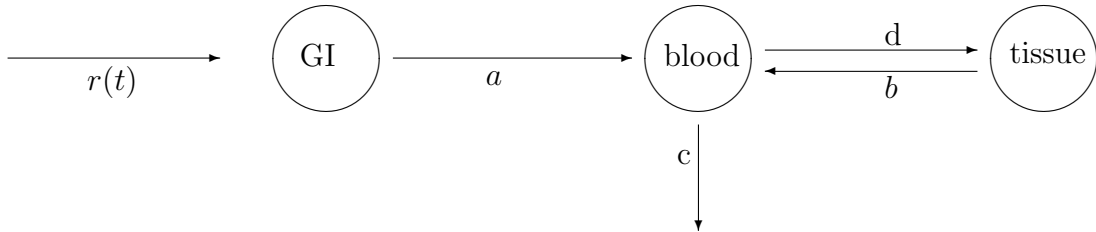


Figure 4: Route to tissue site of infection of an orally-administered drug. Vertical arrow from “blood” leads to metabolism in liver.  $a, b, c, d$  denote flow rates per unit weight of drug.

from vertex 1 to vertex  $n$  in the directed graph  $G(C)$  whose vertices are  $\{1, 2, \dots, n\}$  and where there is a directed edge from  $j$  to  $i$  if  $c_{ij} > 0$ ; otherwise no drug gets to the tissue compartment. See Berman and Plemmons [1]. Let  $r(t)$  be the rate of input of drug into the first compartment. The most common dosing regimes are periodic, at least over some finite treatment time-interval. If  $V_n$  is the volume of the tissue compartment, then  $A(t) = x_n(t)/V_n$  is the concentration of antibiotic at the site of infection.

For example, the matrix  $C$  in the case depicted in Figure 3 where compartment one is GI, two is blood and three is tissue is given by:

$$C = \begin{pmatrix} -a & 0 & 0 \\ a & -d - c & b \\ 0 & d & -b \end{pmatrix}$$

Figure 3 also gives the directed graph  $G(C)$  if one removes the arrow over  $r(t)$ ; note there is a path from the GI to the tissue compartments!

It is easily seen from the variation of constants formula and the positivity of  $e^{Ct}$  that if

$r(t)$  is piecewise continuous and bounded on  $t \geq 0$  and  $x(0) \geq 0$ , then the solution  $x(t)$  of (9) is nonnegative and bounded. Practical dosing regimes have finite duration so  $r(t) = 0$  for all large  $t$ . However, it is still useful to consider the case of constant and periodic dosing for  $t \geq 0$  because most dosing regimes satisfy  $r(t) = \chi_{[0,t_0]}(t)u(t)$  where  $u(t)$  is periodic (or constant) and because the solutions with  $r$  and with  $u$  agree on  $0 \leq t \leq t_0$ . Here,  $\chi_A(t)$  is the characteristic function of set  $A$  and  $t_0 > 0$ . We collect below important properties of solutions of (9)-particularly, we want to know that drug makes its way to the tissue compartment.

**Proposition 3.1** *Let  $C$  be a quasi-positive stable matrix such that there is a directed path from vertex 1 to vertex  $n$  in the directed graph  $G(C)$ .*

*If  $r(t) \equiv r > 0, t \geq 0$  is constant, then all solution of (9) approach the positive equilibrium solution  $x^* = -rC^{-1}e_1 = r \int_0^\infty e^{Ct}e_1 dt$ . Its  $n$ -th component satisfies:*

$$x_n^* = r \int_0^\infty y_n(t) dt > 0$$

where  $y' = Cy, y(0) = e_1$ .

*If  $r(t) \geq 0$  is  $\tau$ -periodic, not identically zero, then all solution of (9) approach the unique  $\tau$ -periodic solution  $x^*(t)$  of (9) and it is positive. It's last component  $x_n^*(t) > 0$  for all  $t$ .*

*If  $r(t) \geq 0$  vanishes for all  $t \geq t_0$ , then every solution  $x(t)$  satisfies  $x(t) = e^{C(t-t_0)}x(t_0)$ ,  $t \geq t_0$  and hence converges exponentially fast to zero. If  $r(t)$  is not identically zero, then  $x_n(t) > 0$  for all  $t \geq t_0$ .*

**Proof:** All assertions follow from the variation of constants formula, positivity of  $e^{Ct}$ , stability of  $C$ , and  $-C^{-1} = \int_0^\infty e^{Ct} dt$ . As noted in [1], there is a directed path from vertex 1 to vertex  $n$  in the directed graph  $G(C)$  if and only if  $[C^m]_{n1} > 0$  for some positive integer  $m$ . Obviously, this implies that  $[e^{Ct}]_{n1} > 0, t > 0$ . ■

The main message of Proposition 3.1 in the case of constant or periodic dosing is that the dose rate-tissue drug level map  $r \rightarrow x_n$  is linear and  $x_n$  can be made large by making  $r$  suitably large.

Our model of antibiotic treatment of tissue infection is

$$\begin{aligned} S' &= d_S(S^0 - S) - F(S)u \\ v' &= d_v(1 - v) - H(u)v \\ u' &= F(S)u - d_u u - \lambda H(u)v - g(S, A)u, \quad A = x_n/V_n \end{aligned} \tag{10}$$

The pharmacodynamic function  $g(S, A)$  can be viewed in two different ways depending on whether the antibiotic kills bacteria or prevents cell division thus slowing growth. In the latter case, it is more natural to modify the growth rate in (10) to  $F(S, A) = F(S) - g(S, A)$ . In the former case,  $g$  represents a death rate. In either case,  $g(S, A)$  should satisfy:

$$g(S, A) \geq 0, \quad g(S, 0) = 0, \quad g_A(S, A) \geq 0 \tag{11}$$

at least on a domain of  $(S, A)$  values of relevance.

Most examples of pharmacodynamic functions used in the literature depend only on antibiotic concentrations [8, 21, 13, 22]. See [16] for a fuller discussion. However, Corpet et al [7] consider nutrient limitation and introduce pharmacodynamic functions which depend on both limiting nutrient and antimicrobial agent. Among several alternatives, they propose combining growth and killing in a single Monod function where either of the two Monod parameters  $m, a$  may depend on antibiotic concentrations. Cogan [5] also considers nutrient limitation in his study of the effect of persister cells on antibiotic treatment, proposing the function

$$g(S, A) = k \frac{S + \alpha}{a + S} A$$

He used Monod bacterial growth rate and notes that when  $\alpha = 0$ ,  $g$  is proportional to growth rate which is consistent with some observations indicating that fast growing cells are more susceptible than slow growing ones to certain antibiotics. When  $\alpha = a$ ,  $g$  is simply proportional to antibiotic concentration, another common choice. Classical  $E_{max}$  theory (see [22, 2, 19]), based on drug and target receptor interaction, posits that the drug effect  $E$  should be a Hill function

$$E = E_{max} \frac{A^h}{A_{1/2}^h + A^h}$$

where  $h$  is the number of drug molecules forming a receptor-drug complex and  $A_{1/2}$  is the antibiotic concentration that illicit half maximum effect. If the killing effect is proportional to growth rate,  $h = 1$ , and  $k = E_{max}$ , then we have the function used in our simulations

$$g(S, A) = k \frac{A}{A_{1/2} + A} \frac{S}{1 + S} \quad (12)$$

Our model of antibiotic treatment consists of (10) and (9). Notice that we neglect any diminution of drug in the tissue compartment ( $x_n$ ) due to its association with bacteria. This is common practice [2, 13, 22] but it may not be accurate. See [15] for inclusion of additional loss terms. As a consequence, there is no feedback of (10) on (9), greatly simplifying the analysis.

We will assume hereafter without further mention that

$$S \leq S^0 \text{ and } v \leq 1. \quad (13)$$

It is easily seen that this region is positively invariant for our system and that any solution starting outside it eventually enters and remains in it. On biological grounds as well it seems unlikely that appropriate initial data would lie outside this set.

The problem is now clear: design a therapeutic dose regime  $r(t)$  that will cure the infection. Of course, there are many constraints that may be relevant to the problem. If the antibiotic is toxic to the host above a threshold dose then the dose may not exceed this level. The antibiotic may have no effect at low concentrations. Also, one would like to minimize the overall dosing time (support of  $r(t)$ ) as well as expense, which is likely to depend on the integral of  $r$  over the dosage time.

There are three cases to consider regarding antibiotic treatment. If  $\Lambda > 0$ , then treatment is necessary since otherwise bacteria persist by Theorem 2.1. We show below that in this case, any finite-time treatment regime fails. If  $\Lambda < 0$  and the infection-free state is globally

attracting then treatment is optional and may speed recovery. Not surprisingly, treatment is always successful in this case. Finally, if  $\Lambda < 0$  but the infection-free state is not globally attracting, then treatment is necessary, at least for a large inoculum of bacteria. We show in this case that it suffices to choose a dosing regime  $r(t)$  that will achieve  $(S(t_0), v(t_0), u(t_0)) \in B$  for some  $t_0$  and then quit treating. At first this may seem obvious since, in comparison with (3), the bacteria now face both immune pressure and antibiotic treatment. This should act to further depress the bacterial population. However, decreasing bacteria has the effect of allowing both nutrient and phagocytes to recover and these two effects counteract each other since nutrient activates bacteria and phagocytes depress it.

Our main result makes these assertions precise.

**Theorem 3.1** *Assume  $\Lambda < 0$  and  $F(S^0) > d_u$ . Let  $r(t) \geq 0$  be any dosing regime such that  $(S(t_0), v(t_0), u(t_0)) \in B$  for some  $t_0 \geq 0$ . Then  $(S(t), v(t), u(t)) \in B$  for all  $t \geq t_0$  and  $(S(t), v(t), u(t)) \rightarrow (S^0, 1, 0)$ ,  $t \rightarrow \infty$ .*

*Assume If  $F(S^0) - d_u - \lambda H'(M) \frac{d_v}{d_v + H(M)} < 0$  where  $M = \frac{d_S S^0}{\min\{d_S, d_u\}}$ . Let  $r(t) \geq 0$  be any dosing regime including  $r \equiv 0$ . Then  $(S(t), v(t), u(t)) \rightarrow (S^0, 1, 0)$ ,  $t \rightarrow \infty$ .*

*Assume  $\Lambda > 0$ . Then there exists  $\epsilon > 0$  such that for any dosing regime  $r(t) \geq 0$  which vanishes for large  $t$ , we have:*

$$\liminf_{t \rightarrow \infty} u(t) \geq \epsilon$$

if  $u(0) > 0$ .

**Proof:** Assume  $\Lambda < 0$  and  $F(S^0) > d_u$ . As in the proof of Theorem 2.1, a solution  $(S(t), v(t), u(t))$  of (10) satisfies

$$\begin{aligned} v' &= d_v(1 - v) - H(u)v \\ u' &\leq F(S^0)u - d_u u - \lambda H(u)v \end{aligned}$$

and (13), from which we may conclude that, if  $(S(t_0), v(t_0), u(t_0)) \in B$ , then

$$1 \geq v(t) \geq V(t) \geq \bar{v}, \quad 0 \leq u(t) \leq U(t) \leq \bar{u}, \quad t \geq t_0$$

where  $(V(t), U(t))$  is the solution of (8) that agrees with  $(v(t), u(t))$  at  $t = t_0$ . This implies that  $(S(t), v(t), u(t)) \in B$  for all  $t \geq t_0$ . Since  $(V(t), U(t)) \rightarrow (1, 0)$ , unless  $(V(t_0), U(t_0)) = (v(t_0), u(t_0)) = (\bar{v}, \bar{u})$ , and since  $(v(t), u(t)) = (\bar{v}, \bar{u})$  for all  $t \geq t_0$  is impossible, we conclude that  $(v(t), u(t)) \rightarrow (1, 0)$ . This forces  $S \rightarrow S^0$ .

If  $F(S^0) - d_u - \lambda H'(M) \frac{d_v}{d_v + H(M)} < 0$ , the proof follows that given in Theorem 2.1 for the same case.

Assume  $\Lambda > 0$ . Since  $r$  vanishes for large  $t$ ,  $A(t) \rightarrow 0$  exponentially fast by Proposition 3.1. Consequently, (10) is asymptotically autonomous with limiting system (3). If  $u(0) > 0$ , the omega limit set  $\omega$  of a solution  $(S, v, u)$  of (10) is a nonempty compact, invariant, chain transitive set relative to (3). See [20] for results for asymptotically autonomous systems. Furthermore, by the same arguments used to prove Proposition 2.1,  $\omega$  must be contained in the box  $[0, S^0] \times [0, 1] \times [0, M]$  where  $M = S^0 \frac{d_S}{\min\{d_S, d_u\}}$ . The only possible point of  $\omega$  on the invariant plane  $u = 0$  is  $(S^0, 1, 0)$  since a backward orbit of (3) through any other point is unbounded yet must belong to  $\omega$ . If  $(S^0, 1, 0) \in \omega$  then  $\liminf_{t \rightarrow \infty} u(t) = 0$ .

But  $u(t)$  cannot converge to 0 since that would imply  $(S(t), v(t), u(t)) \rightarrow (S^0, 1, 0)$  and  $u'(t)/u(t) \rightarrow \Lambda > 0$ , a contradiction. We conclude that  $\omega \not\subseteq \{(S^0, 1, 0)\}$ .

We now apply the continuous time version of Theorem 4.3 of [11] (see Theorem 3 of [28]) where in the notation of the former result,  $X = \mathbb{R}_+^3$ ,  $X_0 = \{x = (S, v, u) \in X : u > 0\}$  and  $M_\partial = X \setminus X_0$ ,  $A_\partial = (S^0, 1, 0)$ . Hypothesis (C.1) of Theorem 4.3 follows from Proposition 2.1 and hypothesis (C.2) is satisfied since  $(S^0, 1, 0)$  is a saddle point whose stable manifold is  $X_0$ . Theorem 4.3 gives the desired result.  $\blacksquare$

In the next subsections, we assume that  $\Lambda < 0$  and  $F(S^0) > d_u$ . The goal is to show that we may choose a dosing strategy  $r(t)$  that will drive  $(S, v, u)$  into  $B$ . Below we give some dosing regimes that will work, depending on the pharmacodynamic function  $g$ .

### 3.1 $g(S, A)$ dominates $F(S)$ for large $A$

If, for example,  $g(S, A)$  dominates  $F(S)$  for  $A$  above some threshold  $A_m$  then we can clearly force  $u$  to decrease. Assuming

$$F(S) - g(S, A) \leq 0, \quad 0 \leq S \leq S^0, \quad A \geq A_m$$

then, by Proposition 3.1, we may choose  $r(t)$  such that  $A(t) > A_m$  and we have

$$u'/u \leq -d_u$$

Obviously,  $u(t) \rightarrow 0$  and it is easy to see that  $v$  approaches 1. Indeed, since there exists  $t_0 > 0$  such that  $H(u(t)) \leq H(\bar{u})/2$  we have  $v' \geq d_v - (d_v + H(\bar{u})/2)v$ ,  $t \geq t_0$  so

$$v(t) \geq v(t_0)e^{-a(t-t_0)} + \frac{d_v}{d_v + H(\bar{u})/2} (1 - e^{-a(t-t_0)}) > \frac{d_v}{d_v + H(\bar{u})} = \bar{v}$$

for large  $t$ , where  $a = d_v + H(\bar{u})/2$ . Therefore,  $(S(t), v(t), u(t)) \in B$  eventually. Clearly this crude approach is far from best possible and it may require toxic or expensive levels of antibiotic to achieve. In case  $g$  is given by (12) with  $k > m$ , then  $A_m = \frac{H}{k-m}$ .

### 3.2 $S \rightarrow F(S) - g(S, A)$ is nondecreasing on $0 \leq S \leq S^0$

Assume that  $S \rightarrow F(S) - g(S, A)$  is nondecreasing on  $0 \leq S \leq S^0$ . If  $r(t)$  is  $\tau$ -periodic,  $x^*(t)$  is the  $\tau$ -periodic solution of (9) as in Proposition 3.1, and  $A^*(t) = x_n^*(t)/V_n$  satisfies

$$F(S^0) - d_u - \lambda H'(M) \frac{d_v}{d_v + H(M)} - \frac{1}{T} \int_0^T g(S^0, A^*(t)) dt < 0 \quad (14)$$

then  $u(t) \rightarrow 0$  as  $t \rightarrow \infty$ . Recall  $M = \frac{d_S S^0}{\min\{d_S, d_u\}}$ .

Indeed, choose  $N > M$  and  $\epsilon > 0$  such that

$$F(S^0) - d_u - \lambda H'(N) \frac{d_v}{d_v + H(N)} - \frac{1}{T} \int_0^T g(S^0, A^*(t) - \epsilon) dt < 0$$

Since  $A(t) - A^*(t) \rightarrow 0$  we can choose  $t_0 > 0$  so that  $A(t) \geq A^*(t) - \epsilon$ ,  $t \geq t_0$ . We argue as in the proof of Theorem 2.1:

$$\begin{aligned} u'/u &= F(S) - g(S, A) - d_u - \lambda \frac{H(u)}{u} v \\ &\leq F(S^0) - g(S^0, A) - d_u - \lambda H'(N) \frac{d_v}{d_v + H(N)}, \quad t \geq t_0 \\ &\leq F(S^0) - g(S^0, A^*(t) - \epsilon) - d_u - \lambda H'(N) \frac{d_v}{d_v + H(N)}, \quad t \geq t_0 \end{aligned}$$

As the mean value of this last quantity is negative,  $u(t) \rightarrow 0$ .

In case  $g$  is given by (12) with  $k \leq m$ , then  $F(S) - g(S, A)$  is increasing for all  $S \geq 0$ .

## 4 Antibiotic Treatment Simulations

We eschew complicated pharmacokinetics in our simulations, essentially assuming the drug is directly introduced into the infection site. This leads to

$$A' = r(t) - d_A A$$

where  $d_A > 0$  is the drug removal rate. We have chosen  $d_A = 1$ .

We have used the pharmacodynamic function  $g(S, A)$  as in (12) with  $k = 0.25$ ,  $d_A = 1$  and  $A_{1/2} = 1$  for all simulations.

In Figure 5 we simulate treatment with constant dosing over the interval  $24 < t < 48$  starting 24 hours after an inoculum of bacteria invades the tissue. We use dosing function

$$r(t) = \chi_{[24,48]}(t)$$

where  $\chi_A$  is the indicator function of set  $A$ .

Figures 7 and 8 simulate the response to discrete periodic doses as depicted in Figure 6. The Matlab function  $r(t) = 5 * (\text{square}(2 * \pi * t/6, 10) + 1) * \text{heaviside}(t - 24) * (1 - \text{heaviside}(t - 72))$ , providing a unit dose every 6 hours over the interval  $24 < t < 72$ , is used in Figure 7 and results in successful treatment. Premature cessation of this regime at  $t = 48$ , shown in Figure 8, results in unsuccessful treatment.

At this stage, having taken more or less arbitrary parameter values for antibiotic levels, our simulations provide at best only qualitative evidence that the model is consistent with expected outcomes.

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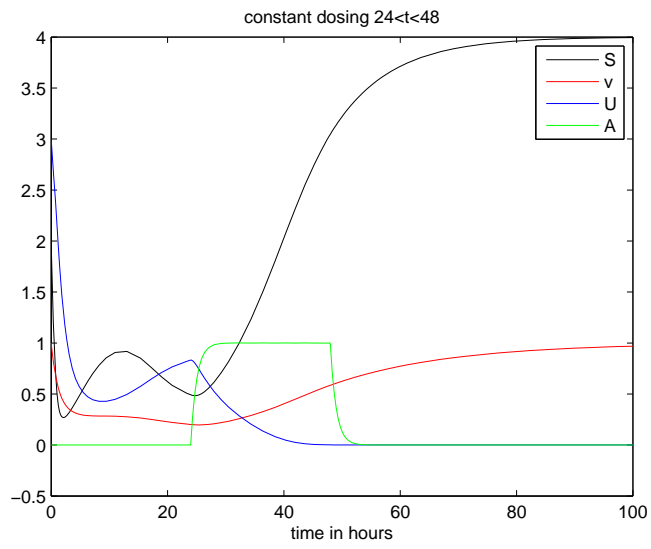


Figure 5: Initial conditions same as bottom Figure 1

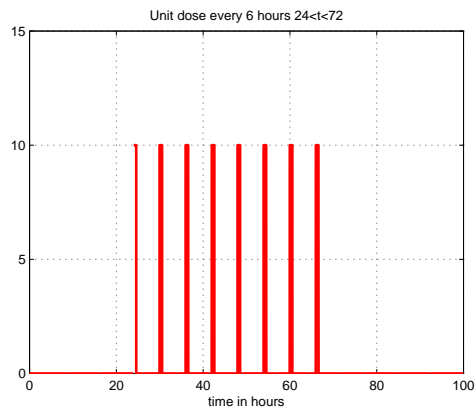


Figure 6: Periodic discrete dosing of antibiotic used in Figure 7

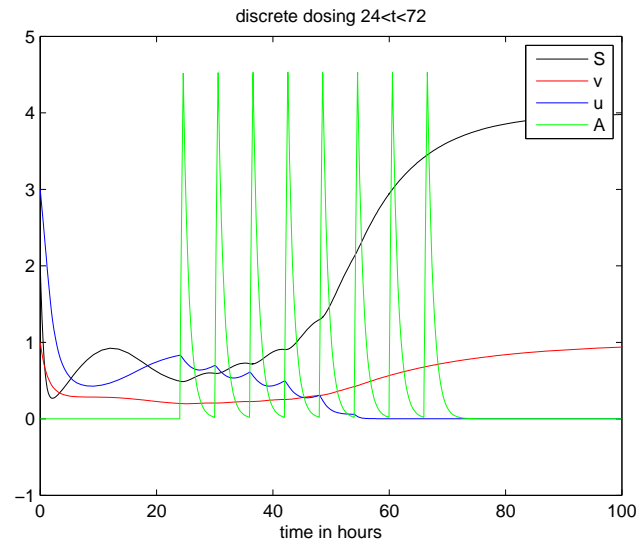


Figure 7: Infection of healthy host followed by successful treatment

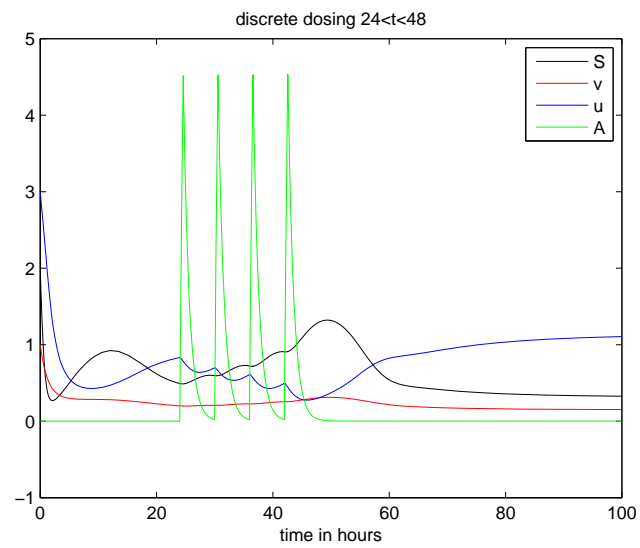


Figure 8: Premature cessation of treatment results in relapse

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