Treatment intensity for pathogens with compound life-cycle

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Abstract: A possible criterion for eventual success of a treatment of a pathogen with a compound life-cycle involves four constants

$$r_{xy}r_{yx} \leq r_{xx}r_{yy}$$

We will firstly consider mathematical consequences of the inequality for an arbitrary pair of integer sequences. These show the necessity of the condition is essentially a tautology. There are diseases when the condition fails to be sufficient but we'll show it can be proved so in cases where a dynamical model gives the correct answer. We relate the condition to existing literature, we give corollaries related to one-way antagonism and induction therapy, and we consider in depth to an example to a hepatitis illnesses where some of the four constants have been calculated already, and we will consider applying these ideas to chronic malaria. The article contains tutorial sections and could be read by medical researchers without mathematical knowledge.

Introduction

While typing this we received a note from nursery that children must be checked for lice egg cases; if found, parents are advised persistent use of a fine-toothed comb. The anecdote is an excuse to motivate the formula which will be described in the article: Let x and y be the numbers of egg cases and lice, respectively, r_{xy} the average rate at the various egg cases produce lice, r_{yy} the average rate at the various lice disappear, and so-on, all measured when x and y take small values x_1 and y_1. A simple counting argument seems to suggest the inequality

$$r_{xy}r_{yx} \leq r_{xx}r_{yy} \qquad (I)$$

should determine whether the regime is sufficiently intense to eventually be successful.

In this article we will consider when it is the case that the truth of the inequality (I) is necessary or sufficient to eventually eradicate a pathogen with compound life cycle, including situations where the physical interpretation of the four constants may be unknown. Firstly, viewed as an abstract mathematical condition on a pair of

integer sequences $x_1, x_2, ...$ and $y_1, y_2, ...$ we will discuss the consequences of truth or failure of (I) on the values of the sequences, showing the necessity of the condition is essentially a tautology. We will discuss illnesses where the criterion is not expected to work, but also verify that (I) is both necessary and sufficient in cases where a dynamical model also gives the correct answer. We call it the *intensity criterion* because in such cases it can determine what intensity of treatment is necessary to eventually eradicate a pathogen with compound life cycle. We give corollaries related to one-way antagonism and induction therapy, relate the condition to existing literature, and discuss in depth the example of a hepatitis illness where some of the four constants have already been calculated. The article includes tutorial sections can be read by researchers without specialized mathematical knowledge.

The condition (I) as a numerical condition.

Suppose we are given two nonnegative integer sequences $x_1, x_2, ..., x_n$ and $y_1, y_2, .., y_n$. These may be measurements of pathogen numbers, or any other sort of measurement. For some reason it may be difficult to directly determine whether x_n or y_n is zero. For example we may have had the ability to detect large values of the sequences and the values of all the terms may be below our favourite detection assay. We want a convenient way of establishing that x_n and y_n are nonzero.

1. Theorem. Suppose $r_{xx}, r_{yy}, r_{xy}, r_{yx}$ are nonnegative real numbers chosen such that the errors in both approximations

$$x_{i+1} \cong x_i - r_{xx}x_i + r_{yx}y_i$$

$$y_{i+1} \cong y_i + r_{xy}x_i - r_{yy}y_i$$

include some positive and some negative values. Suppose the inequality (I) fails, so that the difference $d = r_{xy}r_{yx} - r_{xx}r_{yy}$ is positive. Suppose also the starting values x_1 and y_1 are adequately large, by which we mean $x_1 \geq a/d$ and $y_1 \geq b/d$. Then (regardless, of course, of how large n may be), nevertheless

$$x_n, y_n \neq 0,$$

where the various numbers are defined as follows:

$$\begin{cases} a = r_{yy}E_x + r_{yx}E_y, \\ b = r_{xy}E_x + r_{xx}E_y, \\ E_x = \max_i(-r_{xx}x_i + r_{yx}y_i - x_{i+1} + x_i) \\ E_y = \max_i(r_{xy}x_i - r_{yy}y_i - y_{i+1} + y_i) \end{cases}$$

Proof. If $bx_i \leq ay_i$ then

$$\begin{aligned} bx_{i+1} - bx_i &\geq y_i(-ar_{xx} + br_{yx}) - bE_x \\ &= (y_i d - b)E_x. \end{aligned}$$

Failure of (I) says $d \geq 0$ so the right side is positive as long as $y_i \geq b/d$. If on the other hand $ay_i \leq bx_i$ then $ay_{i+1} \geq ay_i$ as long as $x_i \geq a/d$. In either case the minimum of bx_i and ay_i must increase and can never become smaller than ab/d. The hypothesis that some errors are positive implies that $E_x, E_y > 0$ and therefore that $a, b > 0$ so $ab/d > 0$.

Determining when a dynamical model gives the right answer

As recently as 1995, it was an unanswered question, due to Nigel Burroughs, what sort of equations explain viral loads becoming undetectable and relapsing afterwards. Linear differential equations never do, because they are invariant under scaling. Nor do nonlinear Lotka-Volterra type equations. The theory of nonlinear ordinary differential equations, also called dynamical systems, contains a wealth of such examples abstractly. Research there focusses on finding subtle and mathematically interesting behaviour.

Within the medical literature of the early 1990's the research in virology included measurements of viral loads like

$$130,000 \pm 560,000$$

in which the standard deviation is larger than the mean, and statistical techniques managed to correlate treatment success with rates of reduction early in treatment, for example for HBV and HCV.

However, the correlation was not good. Subsequently, D. Ho et al [6], Xi-Ping Wei et al [7], S. Perelson et al [9] used response to medication to determine coefficients of particular HIV models. The HIV-1 model of [9] involves viral load V, infected CD4 T-cell count I, and total CD4 count $T + I$. This HIV-1 model was not intended to explain relapse after treatment. The mechanism of relapse is believed to relate either to evolution of the virus or to its action on the immune system, which might not be described by any differential equation model.

In [1] the same model is applied to HCV, by replacing CD4 T cells with hepatocytes. There are some differences between the two diseases. The third variable $T + I$ being the total number of hepatocytes, should be just a constant for HCV. For small values of V and I the model is linear and would not be able to exaplain relapse. Also whereas in the case of HIV, infected T cells cannot replicate or come into contact with each other, one has to consider whether hepatocytes might. The slides in [5] show infected hepatocytes distributed randomly in liver tissue rather than clustered, yet the possibility of hepatocyte-to-hepatocyte infection through another route besides serum should be considered. Also, it might actually be the case that there does not exist any ordinary differential equations model, with variables reppresenting serum and hepatic viraemia, which correctly differentiates between sustained response and relapse in hepatitis C. This illness will be considered again later on.

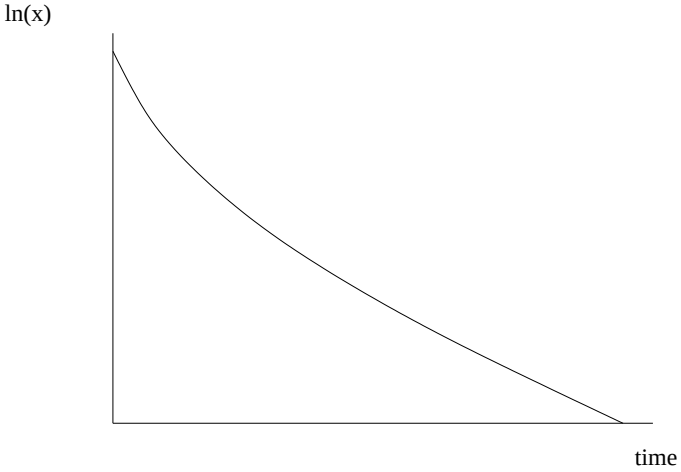
Tutorial about logarithms

Before we proceed further, let us review a familiar use of logarithms. The number x will denote a quantity of pathogen, which could be a count of the number of virions, bacteria, Malaria parasites or malignant cells in a single patient, or the number infected individuals in a population. Typically the natural log $ln(x)$ tends to increase or decrease linearly under various treatment strategies. In medical practise it is more usual to measure x in international units and to use the common log rather than the natural log. Changing the units of x does not affect the rate of change of $ln(x)$ and the common and natural logs are related by the rule

$$ln(x) \cong 2.3\ log(x)$$

Since $ln(x)$ seems to behave linearly, one might try making a graph of $ln(x)$ agaist time. There exists log/linear graph paper for this purpose, in which the vertical axis is already marked in logarithmic units. An optimistic hope would be that if the graph of $ln(x)$ slopes downwards when treatment is commenced, it should eventually pass zero. If this hope were realized, then eventually $ln(x) < 0$ which implies the whole number x satisifies $x < 1$. Then $x = 0$ and the pathogen has been eradicated. Moreover from the slope-intercept formula the amount of time this will take would equal the ratio $\frac{ln(x)}{r}$ where r is the rate of decrease. Such graphs for hepatitis C treatment can be found as early as 1995, and for malaria as early as 2000 [11].

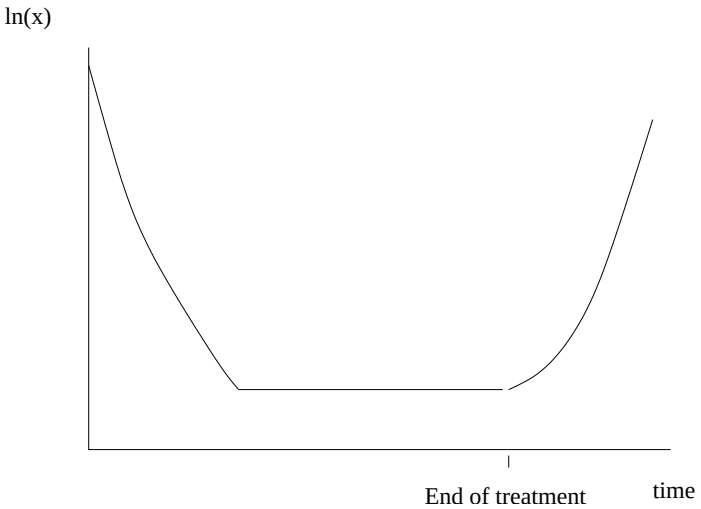
Although there is no mathematical error here, there are two very different circumstances in which the hope would not be realized. The first reason is this: the slope of the graph of $ln(x)$ is the ratio between the time rate of change of x and the value of x. For large values of x the time rate of change tends to be hampered by limiting considerations, but less so as x decreases, leading to worse than predicted success. This particular problem can be solved by letting r be the *limiting* value of the rate of decrease of x as a proportion of x, as x approaches zero.[1]



The more important reason is that often, and invariably in the case

[1] rigorously one has to speak of the upper bound of the limiting set instead of the limiting value

of HIV, x decreases to a nonzero, though possibly very small, stable equilibrium value. Later and later estimates of the limiting value r, appearing to predict treatment success, are really measuring the limit as x approaches a false, nonzero, equilibrium point, and relapse occurs after treatment. The number r, which is limit the as x approaches zero, is not even relevant because x never does approach zero.



There are other possibilities also which occur such as a transient decrease and rebound

Pathogens of a compound life-cycle

Sometimes the existence of a false equilibrium point can be explained by the fact that chronic pathogens, in order to be chronic, need to have a a compound life-cycle. Suppose there is a dynamical model of the pathogen which matches clinical data, in the precise sense that it gives the same answer with regard to the abstract question of eventual treatment success, without regard to treatment duration. Suppose that the model is a two variable model in which the rates of change of x and y are given by well-defined functions of x and y with negative Hessians when considered separately. The hypothesis about the separate Hessians is equialent to saying an attempt to

apply linear extrapolation to one the functions would always lead to an underestimate, possibly due to limiting factors which increasingly take effect for larger x and y. There are now four limiting rates, measured in the matching model

$$r_{xx}, r_{xy}, r_{yx}, r_{yy}.$$

For example, r_{xx} is the time rate of decrease of x as a proportion of x when y is zero, taken in the limit as x tends to zero while r_{xy} is the time rate of increase of y as a proportion of x when y is zero, taken in the limit as x tends to zero. Despite the subscript notation, these are not second partial derivatives of any function r and r_{xy} is rarely the same as r_{yx}.

The only case which needs to be analyzed is where all four numbers are nonnegative, so the treatment is not already obviously inadequate for the variables x and y individually, yet the variables are mutually antagonsitic. We shall assume this is the situation.

2. Theorem. Under these conditions the intensity criterion (I) will match the clinical data on the question whether a treatment strategy is *eventually* effective (with no consideration of the issue of treatment duration).

The proof, which will be given in a later section, also shows that formula (I) controls the position of a single undesireable equilibrium point in the unknown model. When (I) fails but x and y converge, we'll show they must converge to the false equilibrium values and relapse again after treatment is stopped.

Corollaries.

Now we can return to the question of deciding when a suitable dynamical model exists which can match clinical data on eventual treatment success. Both corollaries are negative; i.e., give conditions when no such model could exist. Mathematically speaking, condition (I) is the same as positivity of a two-by-two determinant, which detects when two intersection points of two curves coincide. A different but already familiar condition can be obtained by replacing both r_{xy} and r_{yx} in (I) by the average $\frac{1}{2}(r_{xy} + r_{yx})$. Taking square roots of both sides we obtain just the comparison between an arithmetic mean and a geometric mean, equivalent to positivity

of the determinant of the symmetrized matrix. It is fortunate that the correct intensity criterion did not require symmetrizing the matrix, for condition (I) as it stands has our first interesting corollary about one-way antagonism. If either one of r_{xy} or r_{yx} is zero then the product $r_{xy}r_{yx}$ on the left side of (I) is zero, and the inequality is true. Thus

3. Corollary. Under the hypotheses under which the intensity criterion can be proven, if the two forms of the pathogen are antagonistic but not *mutually* antagonistic (i.e.; one helps the other but not vice-versa), then any treatment intensity which is effective for both separate forms of the pathogen will eventually be effective for the combination of the two forms.

There is another corollary which could be useful in ruling out the existence of a suitable dynamical model.

4. Corollary. If an eventually unsuccessful treatment strategy can converted to an eventually successful one by preceding it by a more intense phase of treatment, one of the hypotheses under which the intensity criterion has been proven must fail.

The corollary is proved by observing that the induction phase just changes the initial values of x and y, yet the inequality (I) which measures eventual success of the second phase does not refer in any way to the initial values; it applies regardless of what they may be.

As examples of ruling out the existence of a suitable dynamical model, firstly, the hypothesis implies the four limiting exponential rates do not depend on the *time* at which x and y can be made to approach zero, relative to start of treatment. In situatins such as microbial resistance the statement of the criterion does not even make sense unless one chooses a putative time of cure at which to measure the four limiting rates. Secondly, there actually are pathogens for which the inequality (I) fails because r_{xx} is not positive, but x can be set to zero during an induction phase and r_{xy} to zero during a maintenance phase. Here the unsatisfied hypothesis is that r_{xx} must be positive.

The case of HCV

An important characteristic of HCV virus is the hypervariablility

of the portion of the genome which controls the lipoprotein envelope, making immune recognition difficult. Accepting that immune recognition is difficult, it is known that interferon treatment alters the situation in an unknown way, and can lead to cure. Yet treatment is not ideally effective and considerations of treatment intensity are necessary. It should be a central concern that, even under treatment, the virus can remain chronic. A number of possible replication sites for the HCV virus have been proposed, including blood mononuclear cells, gut, central nervous system, and liver. Some attempts have been made to measure both positive and negative stranded RNA at these sites. The greatest evidence supports the life cycle described mathematically by Neumann et al [1] in which hepatocytes release virions into the serum in turn infecting hepatocytes. This is an adaptation of the HIV model [9] with CD4 T cells replaced by hepatocytes.

The specific evidence suggesting replication occurs mainly in hepatocytes includes papers starting with an observeed a correlation between interhepatic viral load and serum viral load across patients [3] and papers noting a good correlation also between serum viral load and number of infected hepatocytes [8] and finishing with recent work by [10] who observe in newly infected chimps an early bloom of circulating alanine aminotransferace correlates with spontaneous clearance of the virus.

If we let x be a measure of hepatic viraemia and y the number of circulating virions then some of the four limiting exponential rates are calculated in [1]. The correspondence between notation is given by restating the intensity criterion in the new notation

$$p\beta \leq \delta c \qquad (S)$$

where δ, β, c, p are taken as in that paper, but with appropriate scaling.

Neumann et al suggest administering interferon may act to decrease p or β. There is also presumably the possibility of interferon increasing c by acting on free virions

Herrmann et al [2] calculate some of the coefficients under various treatment strategies, indirectly arguing for example that under pegylated interferon and ribavirin δ is .05 at start of treatment, increasing to .51; that c is 4.7 and p is reduced by .67. They mention

that the effect of ribavirin might be to increase δ. If so, the synergy between interferon and ribavirin is at least consistent with (S), as both medications act to decrease the left side or increase the right side. They indicate that conclusions based on a particular model would need to be backed up by future virological or immunological research.

As we showed in Theorem 1, the necessity of the condition, up to an error margin, is related to a tautology about integer sequences which would have consequences whenever the four constants have any consistent definition whatsoever for small values of x and y. This should not be surprising. There are well-known but simpler numerical tautologies of a similar nature, such as that an end-of-treatment non-responder will not usually be a sustained responder. Regarding sufficiency one might ask about the existence of a dynamical model distinghishing relapse from sustained response. Cases of virological breakthrough would have to be excluded. Without going into any detail, some of the evidence on the side of the existence of a dynamical model are that that immediate retreatment is no less successful than retreatment after a delay, ribavirin acts to prevent relapse without genetic evidence of causing a mutation cascade, the existence of a false equilibrium point is morejconsistent with relapse after undetectability than a resistant quasispecies hypothesis, and so-on.

Other work on the possible effects of ribavirin such as genetic analysis not supporting a mutation cascade, has led to the hypothesis that the effect of ribavirin may be related to immune modulation rather than directly antiviral. This conclusion is tentative and there is debate in the literature about the use of any particular model to measure physical quantities indirectly. (see [4] and related correspondence). One should expect that the HIV model will have difficulties when applied to HCV. As we have mentioned, third variable $T + I$ in the model, which represents CD4 T-cell count in the case of HIV-1, is just the constant number of hepatocytes in HCV. For small values of x and y that particular model becomes linear and would not be able to match clinical data on relapse. And the adapted HIV model does not allow infected hepatocytes to replicate or infect others except via serum. The slides in [5] do show infected hepatocytes distributed in liver tissue rather than clustered and in

this sense it is possible that the failure to allow direct cross infection of hepatocytes may not be as serious as the structural innacuracy of the model as a whole.

Without answering the question whether the particular model used in [1] and [2] is accurate one knows the aim of the analysis in those papers remains useful. The intensity criterion does not require any particular physical interpretation to be given to the four limiting rates. The idea of [2], that ribavirin might act to increase δ, could be tested regardless of whether one thinks the action is related to a decrease of intrahepatic infection – the possibility which was not allowed by the adapted HIV model – or by some other explanation like immune modulation.

One can obtain the four constants directly such as by regression on low values of x and y to minimize the linear errors in Theorem 1 in a least squares sense. Obtaining a sufficient dose to satisfy (S) is a purely pharmacological question, answerable by direct measurement. To do this measurement one needs to measure x and y themselves. It has been possible to directly measure virions within infected hepatocytes at any point of treatment since 1995 [3], and virions in serum since the 1980's. Ignoring ethical questions, one could infect either liver tissue or serum of a healthy volunteer and directly measure the proportional rates of change x and y in each of the two cases.

Ethics presumably prefers measurements while trying to eradicate the pathogen. Also liver biopsies are not ordinarily done just for the sake of virological analysis. Thus the necessity, currently, of the approximate attempts in [1] and [2] and elsewhere to deduce limiting exponential rates using "first slope", "second slope" and "third slope" to approximate to calculating c, δ, and then δ later in treatment, respectivley, and which do need to assume a model, currently the HIV model. Perhaps p could be measured directly using data such as [8] on reapse in a newly transplanted liver, if it were possible to approximate the initial change of hepatic infection. Subject to distortions due to residual effects of medication, perhaps δ could be measured via rapidity of of observed relapse when serum viral load is undetectable but not interhepatic viral load.

There is recent interest in even small fluctuations of ALT during

treatment. This is a measure of hepatic inflammation rather than hepatic viraemia and unfortunately there appears to be little correlation between these [5]. There are linear formulas such as "Actitest" combining a number of liver function tests which have been optimized to measure inflammation. It would be useful to have an analogous regression formula measuring hepatic infection which works during treatment, although no liver function test which has been studied separately correlates in untreated individuals [5] in a two-say correlation.

As the project of measuring the four coefficients, as functions of medication doses, nears completion it will be interesting to compare the inequality (S) actual retrospective data on sustained response versus relapse. The article [2] has already noted a relation between δ and sustained response – but note the measurement there of δ is via third slope, which could also be related to inadequate treatment duration (see the first graph in the section about logarithms above). If the data do not match (I) one will have ruled out in one stroke a range of mathematical models, and this would be a significant contribution to the question of how mathematics may or may not apply. If it becomes difficult to find a statistically significant difference one has a lesser consolation, that the possibility would remain open of applying (S) to diffferentiate between relapse and sustained response.

The case of Malaria.

Malaria shares interesting similarities with hepatitis C, although the pathogen is of a totally different type. Both are thought evade immune detection due to a hypervariable genotype. The two rarer types (vivax and ovale) can have a chronic manifestation in which liver parenchymal cells and red blood cells are infected separately.

Treatment is based on an quinolones, quninie, chloroquine, an extract of the quing hao herb (artemisinin, an asymmetric small molecule with chemical formula $C_{15}H_{23}O_5$ thought to kill circulating Malaria parasites in serum.

The life cycle of Malaria, appears to be that after a person is bitten by a mosquito sporozoites inhabit parenchyma of the liver, each release on the order of 10,000 ring form merozoites into circulation.

Infected red blood cells sequester in the liver spleen or brain, and also circulate. In the two types which can be chronic, also liver cells can be infected.

Even in the two types which cannot be chronic, the malariae and the more dangerous and prevalent falciparum, there is a cycle of illness which since ancient times has been observed. In the case of falciparum there is a fever every two days.

The infection is carried by merozoites which live in circulating red blood cells, and also in red blood cells which become are sequestered in the blood cells of the liver, spleen, or brain.

If we take x and y to be a measure of the sequestered and circulating merozoites, the four numbers involved in the intensity criterion are likely to correspond well with physically measureable parameters.

The article [11] considers that the rising, oscillating graph of falciparum count in relapsed patients can be explained by this compound life cycle of falciparum. The idea is supposed to be that a bloom of infection causes a subsequent bloom in each subsequent generation. In [11] the multiplication factor M represents the total number of infected erythrocytes originating from a single one after a single iteration of the process of generating a sporozyte, so essentially the constant M in that model is the product $r_{xy}r_{yx}$. The distribution $f(t)$ in that paper is described as "similar in shape to the distribution of

merozoites released from the liver over time following hepatic merogony." Because the distribution used is a composite of translates of a single distribution (one translate for each generation considered) the observed syncrhonous oscillation is built into this model.

The intensity criterion is simpler, assuming as it were that the distribution were flat while the sequestered blood cell is alive or present. The width of the distribution then corresonds to the proportional rate at which sequestered parasites perish. The number M equal to the product $r_{xy}r_{yx}$.

The proof for dynamical models.

Now I will turn to the proof that the formula applies in a reasonable range of mathematical models. The assumption is that the rates of change are given

$$dx/dt = f(x, y)$$

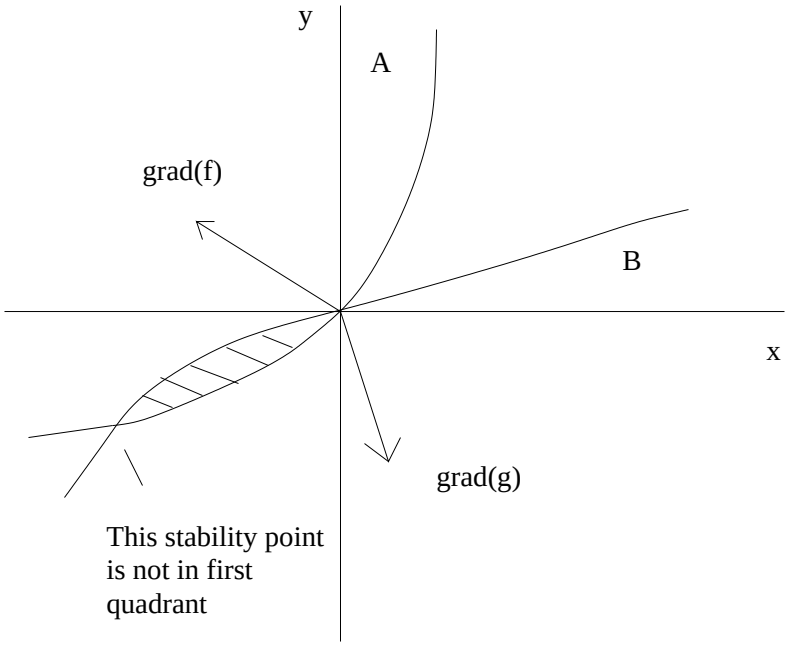
$$dy/dt = g(x, y)$$

where f and g are smooth functions which have negative Hessians when considered separately, and which vanish when $x = y = 0$. From this it follows that the region A where $f \geq 0$ and the region B where $g \geq 0$ are both strictly convex. In other words, a line segment joining two points of the boundary of A is contained in the interior of A and likewise for B.

The gradients of f and g at the origin are $(-r_{xx}, r_{yx})$ and $(r_{xy}, -r_{yy})$. The difference between the two sides of (I)

$$r_{xx}r_{yy} - r_{xy}r_{yx}$$

is just the Jacobian determinant, the cross product of the two gradients. The region where both f and g are positive, being an intersection of convex sets, is again convex, and the geometry of the situation is such that when the determinant is positive, the entire region is contained in the third quadrant.



There may be a second solution of the equation $f = g = 0$ besides the origin, but this must lie on the boundary of the convex region, again in the third quadrant, where x and y are negative. Therefore there is a unique equilibrium point in the first quadrant. This is a universal attractor for the first quadrant. To see this, choose any point (x, y) in the first quadrant. For a, b defined analogously to before, $max(bx, ay)$ decreases over time until $x = y = 0$. This finishes the proof when the determinant is positive. Turning to the case when the determinant is zero we see that either one of A or B is a single point, or else A and B are tangent at the origin and meet nowhere else. In these cases $A \cap B$ is a single point and again meets the first quadrant at only the origin, which is again a universal attractor for the same reason as before. Turning now to the case of negative determinant, we see now that the region $A \cap B$ does meet the first quadrant besides at the origin. For any nonzero point (x, y) in $A \cap B$ in the first quadrant it is now true that $min(bx, ay)$ increases over time whenever x and y are small enough to ensure $(x, y) \in A \cup B$. Certainly the treatment strategy is never effective in this case, and the proof is done.

As promised, let's compare the model when the inequality (I) fails

to real life. If $A \cup B$ covers the first quadrant, we have x and y tending to infinity. In this case the treatment is ineffective as claimed, but in real life nothing tends to infinity, so practically speaking this case does not occur. The meaningfull case, still assuming the determinant is negative, is when $A \cup B$ is not all of the first quadrant. Then the boudnary of A and B meet at two points, and there is a second solution of $f = g = 0$ in the first quadrant besides the origin. This is the promised undesireable equilibrium point which is supposed to explain relapse when (I) fails. Any particular orbit cannot approach zero due to the condition $min(bx, ay)$ increases for small x and y. It cannot approach infinity in real life, as we said. There being just two equilibrium points the orbit must approach the undesireable equilibrium point or become a limit cycle.

I should add, limit cycles being rare in real life (though the behaviour of Malaria is suggestive), one would most often see a situation where x and y would reach the false equilibrium values, possibly being undetectably low. But if treatment were ever discontinued one would see relapse in this case.

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