

Chapter 2

Concepts in Mechanism Based Modeling

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We assume that biological phenomena in principle can be explained in terms of physical and chemical processes. The challenge is 'just' to understand how this can be done.

2.1 Problem Definition

When we talk about biological organisms as systems we refer to the enormous number of mutually reinforcing, competing and counter acting feedback mechanisms that regulate the biological processes in space and time. We cannot hope to capture all aspects of such a highly integrated system in any model. The first and most important step in the biological modeling process must, therefore, be to establish a clear picture of the purpose of the project.

In general terms, the purpose of a modeling effort could be to examine the mechanisms underlying a specific phenomenon and to suggest new critical experiments that could provide insights into these mechanisms. In the health care sector, the purpose could be to design an individualized treatment of a particular disease, and in the pharmaceutical industry the purpose could be to predict parameters

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characterizing the pharmacokinetics of a new drug or to foresee situations where the drug would give rise to adverse side effects. The parameters characterizing the subcutaneous absorption of insulin represent, together with parameters related to the distribution and metabolism of insulin, typical pharmacokinetic parameters.

A clear picture of the purpose of the modeling effort or, in other words, a clear problem definition is required to sort out those processes and interactions we need to take into account from all of those that can be neglected. In most cases this delineation cannot be performed by means of spatial boundaries alone. The kidneys and the liver, for instance, are by themselves complicated systems that support many different processes and when we talk about a heart model, we implicitly refer to particular functions that this organ performs. Other processes that take place in response, for instance, to changes in physical activity, to interactions with the lungs or the nervous system, or to drug administration may or may not be accounted for.

The causal aspects of the problem definition are closely linked to the establishment of a dynamic hypothesis, i.e. a description of how the considered phenomenon can be explained in terms of the proposed mechanisms. This explanation may include a so-called reference mode, i.e. a graph that specifies how the main variables of the system are expected to vary in time, and how this variation is associated with the assumed mechanisms. Specification of the time horizon for the considered phenomena helps us identify processes on one side that develop so fast that they can be considered as displaying a form of quasi-equilibrium and processes, on the other side, that change slowly enough to remain practically constant. Between these limits we find the processes that are essential to the observed behavior.

Most often, an *a priori* delineation of the problem does not exist. On the basis of the phenomena we want to describe, the use intended for the model, the available information, and the time allotted to the project we need to establish a boundary between 'the relevant' and 'the non-relevant'. It may sound, perhaps, as if we suggest solving the problem before we can start to formulate the model. This is, clearly, not the case. However, in view of the enormous complexity we face and the fact that 'everything depends on everything' we have to clarify the problem as well as possible before we are caught up in computer programming and parameter adjustments. In practice, computer simulation, problem definition and model formulation typically develop hand in hand with new aspects and further details being taken into account as the model grows and gains credibility, and as new experiments are performed.

2.2 The Role of Feedback Regulation

Broadly speaking, the feedback structure of a system controls the character of the dynamics it can display and, as mentioned above, one can hardly think of a biological process that does not involve feedback regulation of one kind or another. This underlines the importance of understanding some of the most important aspects

of control theory as developed and used in many areas of engineering. By identifying the main feedback loops of a system, one can often predict the stability of its equilibrium point, the character of its transient response to an external perturbation, and the form of the instabilities it may display.

By far the most common feedback regulation in living systems is negative. This implies that if the value of some variable is changed and we follow the consequences of this change all the way around the causal loop, then the final effect will be a correction that to some extent compensates for the original change.

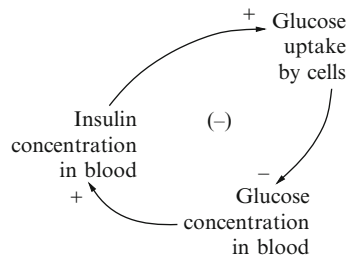
As demonstrated in many books on Classical Control Theory [13, 20] and more recently in books on Systems Biology [17, 28], negative feedback regulation in general ensures stability of the equilibrium point and a reduced sensitivity to parameter variation. This result is particularly important because it allows individual organisms of the same species to exist and function in more or less the same manner while having considerable differences in their parameters. Unless our models reproduce the same feedback structure, the models will clearly fail to represent some of the most essential aspects of the considered systems. In particular, they will fail to display a proper sensitivity to parameter variations. On the other hand, if the model captures the essential feedback structure, the parameters in most cases do not need to be known with very high precision.

Positive feedback regulation also plays a role in the functioning of living systems and may, for instance, be called upon to strengthen or speed-up a particular process. One example is the response of the immune system to an external infection. This response may be seen as a critical race between the infectious agent and the components of the immune system with respect to their ability to proliferate and, for the immune system, to establish an effective defense. Another example is the so-called “calcium-induced calcium release” (CICR) [11, 32]. This is a mechanism that, in response to a small increase in the local cytoplasmic calcium concentration, allows for a rapid growth of this concentration through the release of calcium from intra-cellular stores. A mechanism of this type, involved in the formation of Ca^{2+} microdomains, will be discussed in detail in Chap. 9. Calcium-induced calcium release will also be discussed in connection with the modeling of both smooth muscle cells in the arterial walls (Chap. 10) and heart muscle cells (Chap. 12).

The interaction between glucose and insulin is a classic example of a negative feedback regulation. It is well-known that muscle and adipose tissue cells require insulin to activate receptor molecules in the cell membrane and initiate the processes that will allow glucose to pass into the cells. The higher the insulin concentration in the blood is, the faster glucose will enter the cells, and the more effective the glucose concentration in the blood will be reduced. On the other hand, the blood glucose concentration controls the rate of insulin secretion from the pancreas and, when the glucose concentration decreases, the secretion of insulin also goes down.

Core elements of this feedback are represented in the so-called causal loop diagram of Fig. 2.1. Here, the arrows represent (presumed) causal relations. The signs near the heads of these arrows indicate the direction of change for the effect relative to that of the cause, i.e. plus means that increasing the cause will also

Fig. 2.1 Strongly simplified causal loop diagram for a classic negative feedback: the insulin-glucose regulatory system. The purpose of using this type of diagrams is to clarify the feedback structure of the considered system



increase the effect, and vice versa. The minus sign in parentheses in the middle of the diagram identifies the feedback as being negative. Diagrams of this type are meant to help identifying the basic feedback structure of the system.

In the form depicted in Fig. 2.1, the causal loop diagram only provides a very superficial picture of the insulin-glucose regulation. The diagram neglects, for instance, that brain and nerve cells can consume glucose without insulin, that insulin is degraded both in the liver and in muscle and adipose tissue, and that glucose may be released from the liver. The observant reader will also note that the glucose concentration in the blood does not control the insulin concentration. Glucose concentration determines insulin secretion rate, and this is, clearly, not the same as insulin concentration. It is obvious that we need to develop a clearer picture of “what can be cause” and “what can be effect”.

Nonetheless, a strong negative feedback regulation between insulin and glucose does exist and, in connection with the fast elimination of surplus insulin by the liver, the presence of this feedback leads to the characteristic dynamics of the system, e.g., the characteristic, response to a glucose test with an initial rise in blood glucose concentration followed by a nearly monotonous decay. The life time for insulin in the blood is only 5–8 min, and the fast removal of surplus insulin from the system represents an effective damping mechanism that lends stability to the regulation.

2.3 Flow and Stock Variables

From the point of view of physics, the systems we have described as “cancer cell migration”, “subcutaneous insulin absorption”, and “insulin-glucose regulation” are all thermodynamic systems in the sense that they deal with concentrations and flows. In biology and medicine systems this type are often described as compartmental systems. Conceptually, they represent one of the simplest types of system one can think of.

The description in terms of compartments (or spaces) implies that we can distinguish between two different types of variables: extensive variables and intensive variables. Extensive variables, such as the insulin secretion rate and the amount of glucose in the blood, are characterized by the fact that they scale with the size of the system and can be added for subsystems. Intensive variables are variables such

as the concentration of insulin or the partial pressure of oxygen in the blood. Such variables are independent of the size of the system and do not add for subsystems. The total amount of insulin in the body, for instance, may be obtained by adding the amounts of insulin in the blood and interstitial spaces. By contrast, the insulin concentration in the body cannot be calculated as the sum of the concentrations in the two compartments. We conclude that the mathematical operations allowed for the two types of variables are different.

The extensive variables again divide into stock and flow variables. Stock variables may also be denoted as level (or state) variables, and flow variables as rate variables [12]. All three types of variable are important in our models, and a clear distinction among them helps us understand “what is cause” and “what is effect”: Experimentally one typically measures intensive variables and flow variables, and these two types of variables are often considered as directly responsible for the causal relations of the system. The blood glucose concentration, for instance, determines the rate of insulin secretion, and the insulin concentration controls the rate of cellular glucose utilization. On the other hand, the stock and flow variables together express the material conservation equations that form the most obvious skeleton of the mechanism-based models.

By contrast to the stock variables, concentration variables do not qualify as components of a conservation equation for lumped (i.e., compartmental) systems because they are not related directly to the flows. If, for instance, the amount of insulin in the blood changes we can explain this change in terms of the rate of insulin production in the pancreas, the rate of insulin degradation in the liver and tissue, and the rate of insulin diffusion into the interstitial space. Moreover, the amount of insulin that leaves the plasma compartment and diffuses into the interstitial space equals the amount that appears in that space. By virtue of their different volumes, however, the changes in insulin concentrations resulting from this transfer are not the same.

Admittedly, there are a simple ways to account for this problem, but the situation becomes more complex, if the distribution volumes vary with time. If, for instance, the volume of a particular compartment increases, the concentration in that compartment will decrease, even though there are no out-flow to account for this change. Biological tissue is elastic and yields to pressure changes. This provides many simple examples of time-varying volumes such as the airspaces of the lungs, the ventricles of the heart, the arteriolar and venal volumes, etc.

In accordance with the symbols applied in the field of Systems Dynamics [12] we shall use boxes to denote stock variables, valves to denote rate variables, and circles to denote so-called auxiliary variable. The symbols used for these three types of variables are illustrated in Fig. 2.2. Besides intensive variables as discussed above, auxiliary variables also include stock variables that change fast enough to follow other variables in the system immediately and, hence, can be described in terms of algebraic equations.

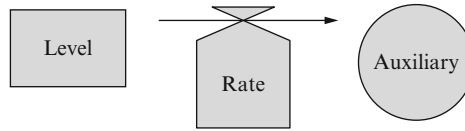


Fig. 2.2 System Dynamics symbols for level (or stock), rate (or flow) and auxiliary variables to be used in the flow diagrams below. The flow diagrams are meant as means to sketch, develop and communicate the main structure of the system. Note that one usually writes the variable names in the respective boxes, valves or circles

2.4 Equations of Motion and the Direction of Causation

Let us assume that we have now chosen the state variables X, Y, Z, \dots of our model. The state variables represent material accumulations that change fast enough for considerable excursions to occur during the time horizon of interest, yet slow enough to display significant phase shifts (or delays) relative to one another. The concept of a compartment is not restricted to be a physical volume. In electrophysiological models of spiking and bursting cells, for instance, a main state variable is the electric charge accumulated on the membrane capacitance. If this capacitance is assumed to remain constant one can, as it is mostly done, use the voltage across the cellular membrane as the state variable.

The stock variables represent the state of the system at any given moment. This state is, so to speak, defined through the amounts of all relevant substances in all specified compartments. The extent to which we want to disaggregate the model, i.e., to divide the different compartments into sub-compartments, depends on the problem and on the purpose of the model. Note, however, that while it may be easy to formally increase the number of stock variables, it is often difficult to obtain the information required to specify how the corresponding flow variables are controlled.

The concept of a deterministic system implies that specification of the state variables X, Y, Z, \dots at a given moment of time makes it possible to calculate the values of all other variables in the system at that moment. In particular, one can calculate the values of the flow variables. Hence, one can determine the change in the state of the system during the next small time interval, and our computer simulations may be viewed as the continued application of this process. All other variables (auxiliaries) of the system are also determined by the state variables, and to start a simulation we only have to provide initial values to the state variables.

For a deterministic (or, more generally, a state determined) system, the equations of motion thus take the form:

$$\dot{X} = f(X, Y, Z, \dots, t) \quad (2.1a)$$

$$\dot{Y} = g(X, Y, Z, \dots, t) \quad (2.1b)$$

$$\dot{Z} = h(X, Y, Z, \dots, t), \quad (2.1c)$$

$$\vdots$$

where t denotes time. The explicit time dependence of the equations of motion allows for possible external influences in the form, for instance, of a sequence of meals or the time profile of some treatment. We generally prefer to minimize the number of external influences in order to explain as much as possible of the observed dynamics by mechanisms that are actually present within the model.

As the above Eqs. (2.1) clearly state, the rate variables are determined by the state variables. We interpret this to mean that the biological cause–effect relationships must be formulated in such a way that changes in the stock variables cause changes in the rate variables. There cannot be cause–effect relationships directly between two stock variables, because a stock variable can only change via in- and out-flows through the associated rate variables. And, there cannot be a direct cause–effect relationship between two rate variables, because the rate variables are determined by the state variables. This again leads us to conclude that every feedback mechanism must involve at least one state variable.

We realize that noise and other forms of randomness are present in most biomedical systems. The model of brain cell synchronization discussed in the section on Parkinson’s disease in Chap. 1 represents a simple example of the significance of such fluctuations. Here, the noise term was used to counteract the drive for synchronization caused by the intercellular interaction. In spite of this ubiquitous presence of noise, however, the present book focuses mostly on the coherent aspects of the observed phenomena.

In this connection it is worthwhile to stress that “noise” has its own generative mechanisms. These mechanisms include thermal excitation of molecular dynamics, random effects associated with a small number of molecules in a given process or space, mutations, biological variation from person to person, etc. Other important contributions arise, of course, from poor control of the experimental conditions, lack of resolution, insufficient sampling rates, etc. Each such source of uncertainty will display its own characteristic properties.

2.5 A Simple Example of a Flow Diagram

In order to illustrate some of the above concepts let us start by discussing a strongly simplified model of the immune response to a virus infection. Let us first make it clear, however, that modeling the immune system and its interaction with foreign agents is an extremely complicated problem that continues to engage a large number of scientists throughout the World [1, 10, 18].

Infection by microorganisms (bacteria, viruses, fungi, etc.) presents a major challenge to all living organisms and, from ants to trees the different species have developed a variety of mechanisms that can help the individual organism overcome the threat of such infections.

Bacterial cells, for instance may be partly protected from viral infections, either because they lack receptors for the specific virus or because they secrete a protective layer that prevents the virus from adsorbing to the cellular surface. Many bacteria

also dispose of a two-component “immune system” consisting of modification enzymes that tag “self” onto proteins produced by the cell itself, together with restriction enzymes that destroy foreign molecules by cutting them into pieces [7]. The viruses on their part can adapt to such resistant cells through so-called host-range mutations that improve their ability to enter the cells or to exploit the cellular functions [4].

Occasionally, a viral DNA may pass unnoticed by the bacterial restriction enzymes and, as the virus starts to reproduce by means of the cellular machinery, the new viruses will be modified by the cellular enzymes so that they can attack other cells with the same restriction-modification system. The viral attack may also take form of a so-called lysogenic response in which the viral DNA is inserted into the bacterial DNA with the result that the cell becomes partially resistant to new attacks. Under stress a lysogenic bacterium may again release its viral DNA, thereby initiating a new round of infection [24].

For vertebrates, the immune system consists of a so-called unspecific (innate) component and a specific (acquired) component [19, 29]. The two components supplement one another such that the unspecific response establishes an immediate defense until the specific and much more effective response can be mounted. The immune system also has a component that remembers past infections and thereby allows the specific response to be established faster and act more effectively in case of a subsequent infection by the same type of virus. As we know from the recurrent flu infections, the virus on its part can mutate such that it will not be recognized so easily by the immune system when it returns in the following season.

The cellular component of the immune response includes primarily the macrophages, the cytotoxic T-cells (CTLs or CD8+ cells), the helper T-cells (HTLs or CD4+ cells), the B-cells and the so-called dendritic cells. These cells all originate from stem cells in the bone marrow. The T-cells derive their name from the Thymus where they are “trained” to distinguish between “self” and “non-self”. In each person, the T-cells are produced in something like 10^9 – 10^{11} different variants (clones), each responding to only a few antigens, but together allowing the body to establish an effective response to a broad range of foreign invaders. During “training” in the thymus, the T-cells are exposed to the normal proteins that circulate in the blood. If a cell reacts to one of these proteins, it is destroyed. Cells that do not react to the body’s own proteins are released to the blood as “mature T-cells”. In the specific response to an infection, lines of T-cells that react strongly to the proteins produced by the infectious agent will multiply particularly fast and thus establish a population of immune cells that effectively can recognize and destroy the pathogen.

Dendritic cells represent the first line of defense. They are found near the external and internal surfaces of the body where an infectious agent will first appear. When a dendritic cell detects the antigens (proteins) of a foreign virus or cell it will travel to a lymph node to activate the T-cells and direct them towards the inflammation site. The macrophages engulf and digest infected cells to present their antigens on the surface. This activates the helper T-cells to proliferate and to stimulate the release of antibodies from the B-cells. Once the infection is over, some of the B- and T-cells will be converted into memory cells. If the same infection later occurs,

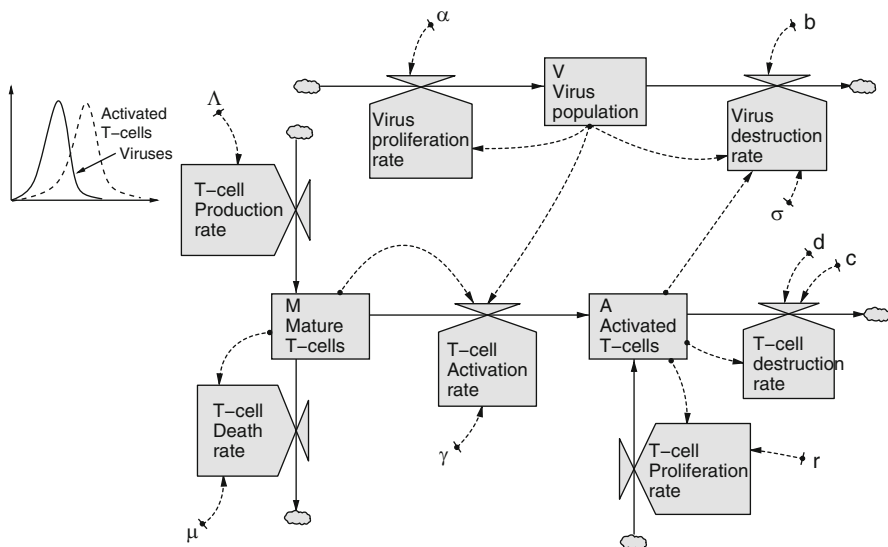


Fig. 2.3 Flow diagram for a strongly simplified model of a virus infection of the immune system. The immune response is described in terms of the activation of T-cells, neglecting all other components of this response

the memory cells are turned back into B-cells that immediately start to produce antibodies against the pathogen. Through the release of compounds known as cytokines, lymphokines, etc., the helper T-cells also stimulate the cytotoxic T-cells to proliferate and to attack the pathogens.

Antibodies, cytokines and lymphokines are elements of the so-called humoral (fluid, i.e., not cellular) component of the immune response. Antibodies bind to the antigens and serve to neutralize the pathogens. Antibodies also bind to the Fc receptor on the T-cells and help these cells locate and destroy the pathogens. Finally, “complement” is the common term for a group of about 20 proteins that normally circulate in the blood in an inactive form. When activated by an infection they stimulate phagocytosis (destruction) of foreign cells, and they may also be directly involved in the destruction of pathogens by making holes in their cellular membrane.

Let us now consider the simplified model of a virus infection shown in Fig. 2.3. This model operates with one state variable V for the virus population and two state variables M and A for the immune system, representing, respectively, the populations of mature and activated T-cells. Neglecting migration of T-cells to other spaces, we may think of these state variables as concentrations of viruses and of mature, respectively activated T-cells in the blood. The fully drawn straight lines that connect the rate and state variables represent the material conservation conditions. The arrows drawn as dotted curves represent the proposed causal mechanisms through which the flows are controlled by the stocks. The parameters (constants) of these causal relations are also indicated.

In accordance with the above discussion, the state variables are determined directly by their initial conditions and the changes they undergo via in- and out-flows through the associated rate variables:

- The number of infectious agents increase through proliferation and decrease through natural death as well as through destruction by the T-cells:

$$\frac{dV}{dt} = \alpha V - bV - \sigma AV \quad (2.2)$$

The parameters α and b are the rate constants for virus reproduction and virus natural death, respectively. By analogy with the (chemical) law of mass action, we have used a simple bilinear term to describe the frequency of cell-virus encounters. σ is the efficiency of virus destruction by activated T-cells. (In the absence of an immune response other mechanisms must be assumed to control the virus population).

- Mature T-cells are released from the thymus at the constant rate Λ . The number of mature T-cells hereafter decreases through natural death with a rate constant (reciprocal lifetime) μ and by activation through encounters with viruses with an efficiency γ . This development is described through the equation:

$$\frac{dM}{dt} = \Lambda - \mu M - \gamma AV \quad (2.3)$$

- Activated T-cells are produced from mature T-cells at the rate γAV and they proliferate at the rate rA . Finally, the activated T-cells are assumed to disappear by natural causes through a combination of linear and nonlinear processes such that in total:

$$\frac{dA}{dt} = \gamma AV + rA - cA - dA^2 \quad (2.4)$$

Note the clear one-to-one relation between the flow diagram and the equations of motion. There is one differential equation for each state variable and it is expressed directly in terms of the flow variables.

The insert in Fig. 2.3 shows a characteristic simulation result with the initial surge of the viral population followed by a rapid decline as the number of T-cells grows large enough. Questions that one could address with this simple model are: (a) With given proliferation and death rates for the virus population what is the minimum reproduction rate and virus destruction efficiency the T-cell population must achieve to cope with the infection? (b) What happens if the nonlinear term in the T-cell death rate is neglected? (c) How can one introduce a form of delay in the T-cell proliferation rate to account for the time it takes for the immune system to develop its specific response? And, considering all other parameters to be known, (d) what is the relation between the T-cell proliferation rate and the duration of the infection?

2.6 HIV Infection of the Immune System

The above infection model can obviously be extended in many different directions. We could try to include some of the most pertinent aspects of the interactions among the infectious agent, the helper T-cells, and some of the other components of the immune system (cytotoxic T-cells, macrophages, antibodies, etc.), or we could try to describe the development of the specific response by allowing for a broad spectrum of different T-cells. Instead, however, we shall try to illustrate how the model, while maintaining its simple structure, can be modified to account for some of the characteristics of an HIV infection.

The human immunodeficiency virus (HIV) is a retrovirus that primarily infects the CD4+ T-cells [2, 6, 30]. The infection is initiated with the adsorption of the virus particle to the cell surface and the binding of the viral gp120 glycoprotein to the CD4 receptor in the cell membrane. This binding causes the viral envelope to fuse with the cell membrane and allows the virus proteins and genetic material to be released into the cell.

The characterization as a retrovirus implies that the genetic material is RNA (rather than DNA). In the cell the viral RNA undergoes a reverse transcription into DNA. This DNA is thereafter inserted into the human chromosome, and new copies of the viral RNA and proteins are produced until the cell finally lyses (bursts), and a large number of new viruses are released. The lifetime of a virus outside the cells is of the order of 6–8 h [27]. The viral DNA may also remain inactive in the T-cell until the cell is activated (e.g. by another infection) and new copies of viral RNA are produced.

The success of the virus in this way hinges on its ability to attack precisely those cells that are meant to orchestrate its destruction. The viral infection even contributes to the proliferation of the CD4+ T-cells, thus increasing the target population. In addition, HIV disposes of a number of mechanisms that allow the virus to evade attacks from the immune system. The inverse transcription from RNA to DNA, for instance, is not controlled as effectively as genetic transcription processes usually are. The result is that the virus mutates relatively often and, if one follows an HIV infected person over a couple of years, one can observe how new and increasingly violent forms of the virus appear from time to time. The fact that the immune system over and over again has to establish a new specific defense may be an important factor in its gradual collapse and the development of AIDS [26].

Another problem is that the gp120 glycoprotein that protrudes from the viral surface is covered by glucose over most of its surface and therefore hard for the immune system to identify as a foreign protein [14]. Only in connection with the binding to the CD4 receptor does the gp120 protein need to open up and reveal its core structure [15].

Figure 2.4 shows a version of our simple infection model, revised to account for some of the most characteristic aspects of an HIV infection. We now have four state variables namely, besides the viral population and the populations of mature and activated T-cells, also a population of infected T-cells. Infection of

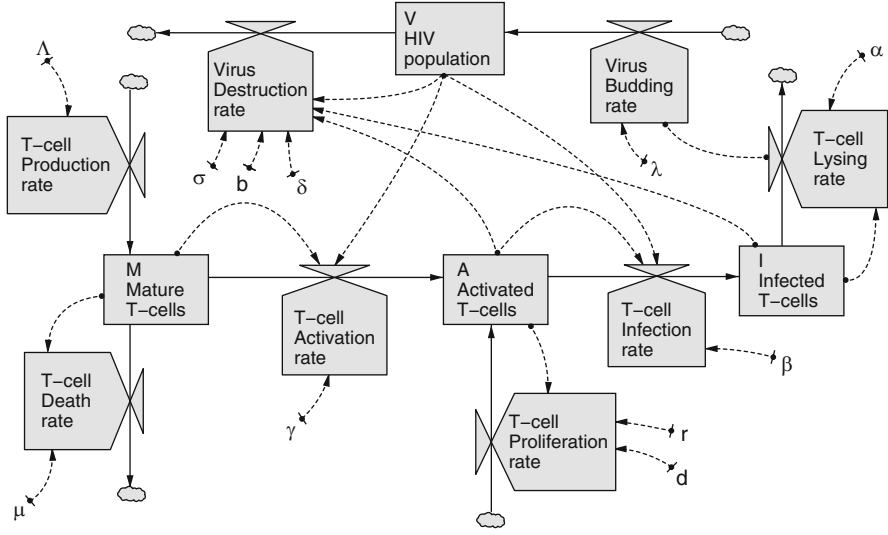


Fig. 2.4 Simplified model describing an HIV attack on the immune system. Note how the virus particles both activate the mature cells to proliferate and infect the activated cells to destroy them. New viruses are released from the lysing cells

the activated T-cells is described by a bilinear term involving the virus population and the population of activated T-cells. The efficiency of the infection process is characterized by the parameter β . Once the infecting virus has reproduced in sufficiently large numbers, new viruses are released through lysing of the cell. The parameter α is now the rate constant for the lysing process, and λ is the burst size, i.e. the number of viruses released in average from a lysing cell. The HIV population is here also assumed to decrease at a rate $\delta(A + I)V$ which represent the rate at which HIV are captured at the surface of activated and infected T helper cells without causing a new infection. With these comments, the following equations of motion can be established directly from the flow diagram [3]:

$$\frac{dM}{dt} = \Lambda - \mu M - \gamma M V \quad (2.5a)$$

$$\frac{dA}{dt} = \gamma M V + r A - \beta A V - d A^2 \quad (2.5b)$$

$$\frac{dI}{dt} = \beta A V - \alpha I \quad (2.5c)$$

$$\frac{dV}{dt} = \lambda \alpha I - b V - \delta(A + I)V - \sigma A V. \quad (2.5d)$$

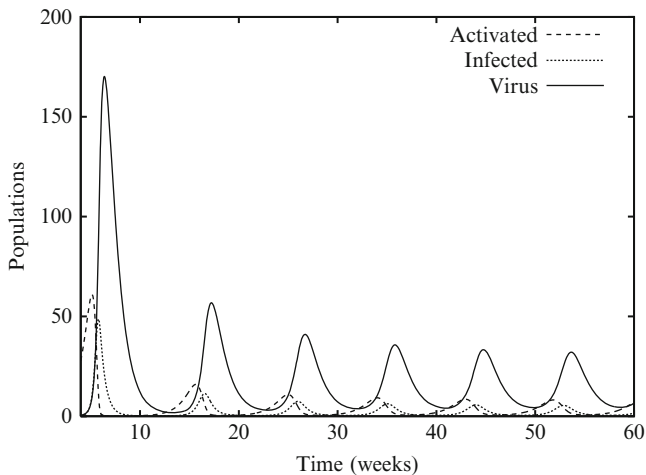


Fig. 2.5 Simulation of the effect of a HIV infection of the immune system. Note the initial upsurge in the viral population, before an effective immune response can be established. Note also the self-sustained oscillations with a period of approximately 10 weeks

Figure 2.5 shows an example of a simulation with the model. Here we have used the parameter values [2]:

$$\begin{aligned} \Lambda &= 1.0, & \mu &= 0.1, & \gamma &= 0.01, & r &= 1.0, & \beta &= 0.1, \\ d &= 0.001, & \alpha &= 2.0, & \lambda &= 5.0, & b &= 1.0, & \sigma &= 0.1. \end{aligned}$$

All concentrations are normalized (and dimensionless). This implies, of course, that no absolute information about concentrations etc. can be drawn from the simulations, and only the produced dynamics can be examined. The time scale is in weeks. As before, the parameter σ measures the efficiency at which the activated T-cells destroy the virus.

When inspecting the simulation we first observe the initial upswing in the virus population until the specific immune response is established. However the virus population is never fully wiped out and the system continuous to display periodic surges in the viral population accompanied by oscillations in the T-cell count as new viruses are released from the infected cells. Sustained oscillations of this type arise through instability in the feedback system. This instability is related to the time delay and the significant gain factor associated with the virus reproduction process. However, it is a system's property and its occurrence depends on several other factors as well. We shall provide a more detailed discussion of the mechanisms involved in this type of instability in Chap. 4.

2.7 HIV Vaccination and Anti-Retrovirus Therapy

The purpose of a vaccination is to stimulate the immune system in such a way that it exhibits a fast and specific response to a given pathogen. Once the immediate stimulatory effect of the vaccination has died out, some of the B- and T-cells are converted into memory cells and, if a real infection later occurs, the memory cells will be turned back into B-cells and start to produce anti-bodies to neutralize the pathogen and assist the immune cells destroy it. Unfortunately, despite very significant efforts over the past 30 years, the interaction between HIV and the human immune system has proved to be too intricate for a successful HIV vaccine to be developed and, today it is probably correct to say that the pharmaceutical industry is reluctant to undertake the risks and enormous costs associated with new attempts at developing an HIV vaccine.

The fact that the virus enters and hides in the human cells makes the development of an anti-virus vaccine very difficult, and only relatively few such vaccines exist. It is well-known, however, that it has been possible to develop an anti-HIV therapy that is capable of effectively suppressing the infection and maintaining it at a very low level for many years. In this way one can stop the weakening of the immune system and it is even possible for the immune defense to recover from damage it has already suffered.

To avoid that the virus develops resistance against the treatment, the anti-retrovirus therapy is usually based on a combination of three or more drugs. The idea is to choose a cocktail of drugs that together inhibits several of the processes that are essential to virus infection and reproduction. To the extent possible, this cocktail should be designed in such a manner that a virus that escapes the inhibitory mechanisms of one drug will have a reduced chance of also becoming resistant towards one of the other drugs.

The individual drugs in the combination therapy may interfere with the functions of the HIV protein (reverse transcriptase) that direct the reverse transcription from RNA to DNA or they may inhibit enzymes (protease) that the virus needs for its reproduction. Other classes of drugs work by reducing the ability of the virus to enter the immune cells or to insert its genetic material into the cellular genes. Nearly 25 such anti-HIV drugs have been approved by the regulatory authorities (The US Food and Drug Administration, FDA), but availability of these drugs is not the same in all countries. The relatively large number of different drugs makes it possible in general both to avoid severe side effects and to establish a second line of defence if the virus becomes resistant to the first drug combination.

One of the sources of complexity that the development of a useful HIV-vaccine must overcome is associated with the ambiguous relation between HIV, antibodies and the CD4+ T-cells [8]. As mentioned above, the gp120 protein on the virus surface binds to the CD4 receptor in the cell membrane, and this contact allows the virus to unload its genetic material and a few essential proteins into the cell and take control of its reproductive machinery. Antibodies against HIV bind to the gp120 antigen and thereby reduce the number of active sites. Each HIV particle carries of the order of 90 gp120 molecules. One can therefore conclude that the

antibody concentration required to completely neutralize the viruses must be at least a hundred times larger than the virus concentration, and presumably significantly higher to account for a majority of antibodies that do not bind to the virus protein. A more precise estimate depends on both the binding affinity and the absolute concentrations.

Antibodies against HIV also bind to the Fc receptor in the cellular membrane, and this allows the T-cell to hold and destroy the virus. However, this type of binding may promote contact between the gp120 protein and the CD4 receptor and in this way enhance the probability of a virus infection. The net effect of these two competing processes will depend on the binding affinities, but also on the relative concentrations of antibodies, gp120 proteins, and T-cell receptors. One would expect, however, that an antibody-dependent enhancement of the infection rate could occur at intermediate concentrations where the number of antibodies is insufficient to effectively neutralize the gp120 proteins and yet high enough to promote contact between the virus and the CD4 receptor. The actual situation is further complicated by the fact that complement may increase the adhesion of the virus to the target cell by binding to both the virus and the CD2 and CD3 receptors in the membrane.

The immediate questions to present themselves are: (a) how significant can such an enhancement be and (b) how large an antibody concentration is required to effectively neutralize the gp120 proteins and avoid the enhancement. The answers to these questions depend on the values of different rate constants and binding probabilities including the rate at which virus particles collide with the surface of an immune cell and the probability that a virus particle binds to either an Fc receptor or a CD4 receptor. Estimates of this type of parameters may often pass as substitutes for real data until experiments can be performed. Let us therefore try to illustrate how such estimates can be performed.

The maximum rate k_d of diffusion limited aggregation may be obtained from the so-called Smoluchowski equation [9]

$$k_d = 4\pi(D_c - D_v)(r_c - r_v), \quad (2.6)$$

where D and r are the diffusion constant and the radius for the cells (index c) and the viruses (index v), respectively. This equation gives us the factor that, multiplied by the two concentrations, determines the number of virus-cell collisions per unit time and unit volume in the random diffusion of the two types of particles. The diffusion constant for a particle of radius r is given by

$$D = \frac{k_B T}{6\pi\eta r}, \quad (2.7)$$

where k_B is Boltzmann's constant, T the absolute temperature and η the viscosity of blood (or of the suspension in which the experiment is performed). In view of the fact that the cellular radius is about 70 times the viral radius, the expression for k_d reduces to only depend on this ratio and a few more general parameters.

The parameter k_d relates to a diffusion process that takes place in three-dimensional space. Once the virus has arrived at the surface of the cell, the motion is reduced to something like a two-dimensional diffusion, and the probability that the virus will find a receptor to bind to may be estimated from [5]

$$p_a = \frac{Nr_s}{Nr_s + \pi r_c} \quad (2.8)$$

Here, N denotes the number of receptors on the surface of the cell, and r_s is the functional radius of the receptor, i.e., the distance within which the virus has to pass to actually bind to the receptor. This distance is considered to be of the order of 1 nm. As before, r_c is the radius of the cell.

In the present case we have two different receptors on the cell surface (CD4 and Fc), and different parts of the virus surface are covered with different ligands: either gp120 that will bind to the CD4 receptor or antibody-complexed gp120 that will bind to the Fc receptor. In this case, the expression for the binding probability may be generalized to read [21, 22]

$$p_a = \frac{(1 - f_a)N_{CD4}r_s + f_aN_{FC}r_s}{(1 - f_a)N_{CD4}r_s + f_aN_{FC}r_s + \pi r_c}, \quad (2.9)$$

where f_a is the neutralization factor, i.e., the antibody-complexed fraction of gp120 molecules on the virus surface. N_{CD4} and N_{FC} denote the numbers of CD4, respectively Fc receptors on the cell surface. If binding of antibodies to gp120 does not block the binding of gp120 to CD4 (i.e., in the case of negligible antibody neutralization), the factor $(1 - f_a)$ in front of N_{CD4} should just be 1. For simplicity, the expression for p_a assumes that the two receptors have the same functional radius, and that the virus surface in practice is completely covered by gp120. (If these assumptions are not correct, one can easily amend the expression for p_a by introducing factors that account for the “active” fraction of the virus surface and for the ratio of the two functional radii). The remaining problem is now to estimate the antibody-complexed fraction of gp120 molecules. Here we have to consider the chemical balance between binding and dissociation of antibodies and gp120 molecules. Considering the reaction to take place between gp120 and antibodies in a suspension, rather than on the virus surface, we may write

$$f_a = \frac{K_m[\text{Ab}]}{1 + K_m[\text{Ab}]}, \quad (2.10)$$

where $[\text{Ab}]$ is the free antibody concentration and K_m the equilibrium constant for the binding-dissociation reaction (i.e., the ratio of the forward to the backward reaction constants). Unfortunately, K_m is not known with any significant accuracy. We have therefore decided to make use of the functional form of (2.9), but to fit the calculated enhancement curve to the experimental results obtained by Takeda et al.

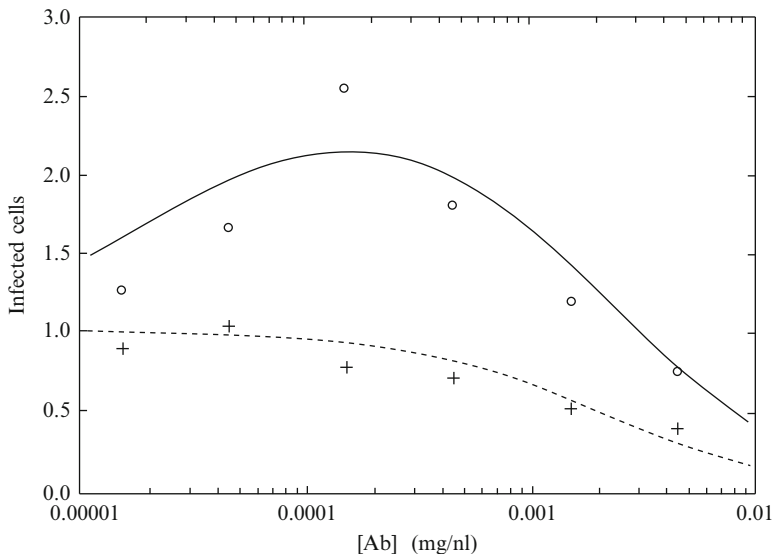


Fig. 2.6 Antibody enhancement of HIV infection: Number of infected T-cells relative to the cells infected in the absence of antibodies as a function of the antibody concentration. *Full and dotted curves* are simulation results for intact antibodies and for antibody fragments, respectively. *Diamonds and crosses* are experimental results obtained by Takeda et al. [31]

[31]. This gives a value for K_m of 10^9 M^{-1} , or a factor of two above the highest value given in the literature.

Figure 2.6 shows how the above model can reproduce the results of the experimental study of antibody enhanced HIV infection performed by Takeda et al. [31]. In this fit it was assumed that antibody-complexed gp120 can bind to CD4 receptors, but that the complex is unable to infect the cell. The simulation model used to reproduce the experimental procedure was initiated with $5 \cdot 10^5$ uninfected monocytes and 10^4 viruses per ml suspension. After 2 h, the number of free virus was set to zero to mimic the washing out of virus particles often performed in HIV infectivity studies and the simulation was continued until time 48 h.

The purpose of the above discussion has not been to carry the analysis through in all detail, but to illustrate how parameter estimation can contribute to the clarification of a problem and, perhaps, reshape the requirement for experimental data.

2.8 Gene Therapy of AIDS

In the absence of a useful vaccine, gene therapy of HIV is often considered a possible alternative. The idea of this approach is to insert anti-viral genes into the patients CD4+ T-cells in order to make them more resistant to the virus attacks.

This resistance may be achieved in different ways such as, for instance, protection of the cells against virus penetration through the cellular membrane, suppression of virus proliferation through genes that inhibit the insertion of viral DNA into the cellular genes or interfere with the production of essential viral proteins. Most of the possible points of attack are, clearly, identical to those of the combination treatment. The difference between the two approaches is that where gene therapy intends to remove the causes of the disease, the combination treatment only relieves the symptoms. In practice this means that whereas gene therapy can be performed once and for ever, the combination treatment requires that the patient day after day adheres strictly to a number of restrictions.

The technical difficulties associated with the development of a gene therapy are, obviously, quite challenging [25]. In any specific attempt, the target process has to be selected (i.e. which part of the HIV infection-reproduction cycle should one try to inhibit), and a vector has to be chosen (i.e. what means should be used to insert of the new genes into the cells). The most common choice of a vector is a virus that is known to attack human cells and exploit their genetic machinery. However there are many different types of virus and one has to consider (a) if the virus attacks the targeted cells, (b) if it inserts the resistant gene in a stable and consistent manner, (c) if it is easy to manipulate in the laboratory, (d) if it causes any disease or produces a response from the immune system, (e) if it presents a threat to the descendants of the patient or to the population in general, etc.

Next the virus has to be manipulated so that much of its own genetic material (particularly those parts that may cause disease or immune response) is removed and replaced by the genetic material to be transduced to the human cells, and a comprehensive series of laboratory experiments have to be performed to demonstrate the possibility of the suggested process and its adherence to all safety requirements.

At the present very few clinical experiments have been performed (presumably less than 1,000 for AIDS patients). A few successful experiments have been reported where stem cells from an HIV resistant donor have been transplanted into the bone marrow of HIV-patients with a life-threatening condition such as leukemia [16]. In the cited case the donor cells have displayed a genetic mutation that inhibits expression of the co-receptor CCR5, a protein that, together with the CD4 receptor, is required for HIV to penetrate into the CD4+ T-cell. Fifteen to twenty percent of the North-European population is thought to carry this mutation on one allele while 2%–4% has inherited the mutation from both parents and, therefore, is resistant to HIV. Although stem cell transplantation differs from gene therapy, the positive result of the above treatment supports the notion that the CCR5 co-receptor plays a critical role in HIV infection, and that this receptor could be the target for gene therapy.

Another set of questions relate to how effective the protection of the transduced cells has to be for the gene therapy to be effective and how this is related to the aggressiveness of the virus. One may also ask if it is necessary to treat the T-cell progenitor cells in the bone marrow or if treatment of the mature T-cells in the blood suffices. In the latter case, the treatment may perhaps be performed simply by taking a blood sample, inserting the protecting gene into the chromosome of the CD4+ T-cells, and re-infusing the blood into the patient. After a while, the population of

non-protected CD4+ T-cells will be significantly reduced due to virus attacks, and the resistant CD4+ T-cells will have spread throughout the blood and into others compartment of the body (e.g., the lymph nodes). A problem is that matured T-cells will continue to be supplied from the thymus, and it is unlikely that these cells will be protected, unless the stem cells are also transfected.

To examine these questions we may use the following simple model [23]. We consider the dynamics of two different populations of CD4+ T-cells, a population T of normal (i.e., non-transduced) CD4+ T-cells described by the equation:

$$\frac{dT}{dt} = (1 - f)\Lambda_c + T\lambda_c(T_t) - (\delta_T - \delta_H)T \quad (2.11)$$

and a population T' of transduced CD4+ T-cells described by:

$$\frac{dT'}{dt} = f\Lambda_c + T'\lambda_c(T_t) - (\delta_T - (1 - \eta)\delta_H)T' \quad (2.12)$$

Here, $T_t = T + T'$ is the total T-cell population. Λ_c is the rate of supply of mature CD4+ T-cells from the thymus, and f is the fraction of the hematopoietic progenitor cells that is transfected. $f = 0$ means that only cells in the blood are transfected whereas $f = 1$ means that all T-cells including those released from the thymus are transfected. δ_T is the normal death rate constant for T-cells in the absence of HIV and δ_H is the additional death rate constant caused by the HIV infection. η is the degree of protection against HIV-promoted deaths that the transfected T-cells have acquired. This formulation implies that we do not necessarily assume that transfected cells are completely protected. Finally, $\lambda_c(T_t)$ is the growth rate function for which we shall assume the Monod-like form

$$\lambda_c(T_t) = \frac{a}{b + T_t} \quad (2.13)$$

As we use it here, this form implies that the saturation features of the cell proliferation are shared between the two cell populations. The normal death rate constant is assumed to be $\delta_T = 0.01$ day, corresponding to a natural life time for the T-cells of 100 days. In a state of equilibrium, the death of T-cells by natural causes is compensated by a supply of new mature T-cells from the thymus and by a relatively weak proliferation of the T-cells. Let us assume that the equilibrium T-cell count is 1,000 cells/mm³, that 20% of the T-cells originate directly from the thymus, i.e. $\Lambda_c = 2$ cells/mm³/day and that the remaining cells originate from cell proliferation, i.e. $\lambda_c(T_t) = 8$ cells/mm³/day. These numbers correspond approximately with experimental conditions where one finds normal T-cell counts in the interval 500–1,200 cells/mm³. For a half-saturation value in the Monod equation of $b=1,000$ cells/mm³, this finally gives us a value for the maximal rate of T-cell proliferation of $\lambda_c(1) = 16$ cells/mm³/day.

With these parameters we can now draw the curve sets shown in Fig. 2.7a, b for $f = 1$ and $f = 0$ respectively. We recall that f measures the fraction of

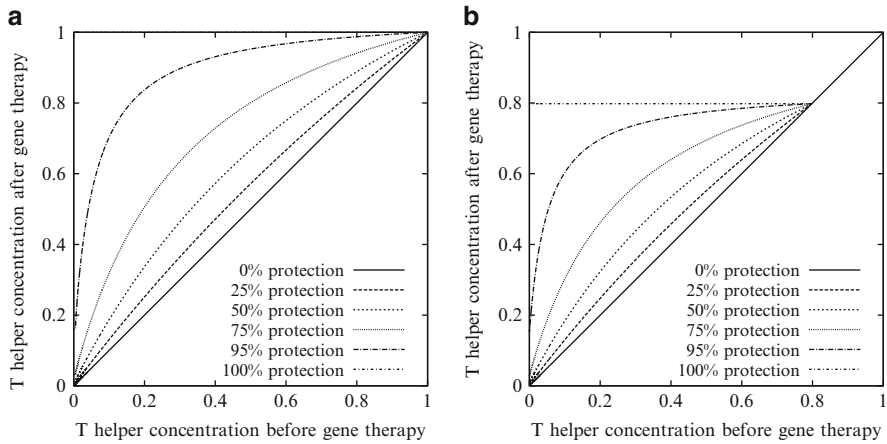


Fig. 2.7 Normalized concentrations of T-helper cells at steady state after gene therapy as functions of the normalized concentration before therapy. The parameter represents the degree of protection that the gene manipulated T-cells have acquired. **(a)** Gene therapy affects the stem cells in the bone marrow ($f = 1$). **(b)** Gene therapy affects the mature T-cells only ($f = 0$)

transduced stem cells. In both figures, the horizontal axis shows the CD4+ T-cell concentration before the gene therapy, and the vertical axis shows the CD4+ T-cell concentration after the treatment. Both concentrations are normalized by division with the assumed equilibrium concentration in the non-infected person (1,000 cells/mm³). The parameter that determines the position of the system along the horizontal axis is the HIV inflicted death rate δ_H , and the parameter that separates the individual curves in the two plots is the degree of protection η for transduced cells.

For $f = 1$, 100% protection (of all the cells) obviously means that the T-cell population after the gene therapy reestablishes the normal value for an uninfected person irrespectively of the aggressiveness of the virus. As the degree of protection is reduced, so is the T-cell population, and this reduction is particularly strong to the left in the figure where the viruses are very aggressive. For 90% protection, the T-cell population remains above 600 cells/mm³, even under conditions where the untreated person would have cell counts as low as 50 cells/mm³. Without protection of the stem cells, the attained cell count is typically 20% lower. In particular, the cell count for 100% protection of the mature T-cells never exceeds 800 cells/mm³ if the untreated person has a lower cell count.

As mentioned above, the normal T-cell count for a non-infected person is of the order of 1,000 cells/mm³. Over the years, the T-cell count for an HIV-infected patient gradually declines as the immune system weakens and the viruses tend to become increasingly virulent. Initiation of a combination treatment as described in Sect. 2.7 is usually decided when the T-cell count falls below 350 cells/mm³. The viral load, i.e. the number of virus copies in the blood is another important

parameter. If the combination treatment works as intended, the viral load should be below 50 virus/ml.

2.9 Conclusion: Model Formulation

The interconnectedness and unusual degree of integration of biological processes poses a specific challenge to the modeling of biomedical systems. Today's engineering systems may also be complex, but we have designed them ourselves, we know how they work, unnecessary coupling between processes that take place at different time scales has been avoided, and the spatial structure is in most cases reasonably logic. The same conditions are not satisfied in the living world. This requires that we are very conscious about the processes of problem definition and system delineation. The boundaries of the system in space and time are not self-evident, but have to be decided in relation to the defined problem. This again depends on the purpose of the modeling effort and the degree of detail to be achieved.

The use of flow diagrams to represent the structure of a biological system may be seen as a typical engineering approach. It derives from the need to easily overlook the interplay between different components and interactions and represents the conception that "structure generates behavior" [12]. The use of such diagrams in Systems Biology may also be viewed as a way to generate a common ground between an often empirically oriented MD or biologist and a modeler with a focus on mathematical equations and simulation methods. As the model grows in size, the flow diagrams tend to become too complicated to serve their purpose as means of communication. They may still be very useful, however, in the structuring of separate parts of a large model.

Besides the detailed structural representation provided by the flow diagrams with their material conservation conditions and explicit distinction between different variable types, we have also illustrated the use of the somewhat simpler causal loop diagrams. These diagrams may be applied in the first attempt to structure the cause–effect relationships of a new problem or they may be used to clarify the main feedback interactions in a fully developed model. A main weakness of the causal loop diagrams is that they do not provide a guide as to the direction of the cause–effect relations. This is precisely the guidance that the flow diagrams provide by maintaining that changes in the value of a state variable cause changes in the flow variables.

A model that doesn't include feedback loops is unlikely to capture essential aspects of a living system. If the dominant feedback is negative (correcting feedback), the system may display a monotonous approach to the equilibrium point in response to external perturbations or, if the equilibrium point display complex conjugated eigenvalues, a damped oscillatory response. If the feedback gain is high enough, or the delay in the feedback long enough, the equilibrium point may become unstable. The system may then display growing oscillations or, in the presence of nonlinear restrictions, a self-sustained oscillatory behavior may occur.

If the main feedback loop is positive (reinforcing feedback), the model tends to produce exponential growth, extinction of parts of the system or, in the presence of nonlinear constraints, coexisting equilibrium states, hysteresis, boom-bust dynamics, bobbles, etc.

References

1. Althaus CL, De Boer RJ (2008) Dynamics of immune escape during hiv/siv infection. *PLoS Comput Biol* 4:e1000103
2. Anderson RM, May RM (1988) Epidemiological parameters of HIV transmission. *Nature* 333:514–519
3. Anderson RM, May RM (1989) Complex dynamical behaviour in the interaction between HIV and the immune system. In: Goldbeter A (ed) *Cell to Cell Signalling, From Experiments to Theoretical Models*. Academic Press, New York
4. Arber W (1965) Host-controlled modification of bacteriophage. *Annual Review of Microbiology* 19:365–377
5. Berg HC, Purcell EM (1977) Physics of chemoreception, *Biophysics Journal* 20:193–219
6. Berger EA, Murphy PM, Farber JM (1999) Chemokine receptors as HIV₁ coreceptors: roles in viral entry, tropism, and disease. *Annual Review of Immunology* 17:657–700
7. Bull AT, Slater JH (1982) *Microbial Interactions and Communities*. Academic Press, New York
8. Burke DS (1992) Human HIV vaccine trials: Does antibody dependent enhancement pose a genuine risk? *Perspectives of Biological Medicine* 35:511–530
9. Chandrasekhar S (1943) Stochastic problems in physics and astronomy, *Review of Modern Physics* 15:1–89
10. De Boer RJ (2007) Understanding the failure of cd8+ t-cell vaccination against simian/human immunodeficiency virus. *J Virol* 81:2838–2848
11. Fabiato A, Fabiato F (1977) Calcium release from the sarcoplasmic reticulum. *Circulation Research* 40:119–129
12. Forrester JW (1961) *Industrial Dynamics*. MIT Press, Massachusetts Institute of Technology
13. Franklin G, Powell JD, Emami-Naeini A (1996) *Feedback Control of Dynamic Systems* (4th edition). Prentice Hall, New Jersey
14. Hansen JE (1992) Carbohydrates of human immunodeficiency virus. *APMIS* (suppl. 27) 100:96–109
15. Hansen JE, Lund O, Tolstrup N, Gooley AA, Williams KL, Brunak S (1998) NetOglyc: Prediction of mucin type O-glycosylation sites based on sequence context and surface accessibility. *Glycoconj J* 15:115–130
16. Huetter G, Nowak D, Mossner M, Ganepola S, Mussig A, Allers K, Schneider T, Hofmann J, Kuecherer C, Blau O, Blau IW, Hofmann WK, Thiel E (2009) Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation, *New England Journal of Medicine* 360: 692–698
17. Iglesias PA, Ingalls BP (1996) *Control Theory and Systems Biology*. CRC Press, New York
18. Iwami S, Nakaoka S, Takeuchi Y (2008) Viral diversity limits immune diversity in asymptomatic phase of hiv infection. *Theor Popul Biol* 73:332–341
19. Janeway Jr CA, Travers P, Walport M, Shlomchik MJ (2001) *Immunobiology - The Immune System in Health and Disease*. Garland Science, New York
20. Levine WS (1996) *The Control Handbook*. CRC Press, New York
21. Lund O, Hansen J, Mosekilde E, Nielsen JO, Hansen J-E S (1993) A model of enhancement and inhibition of HIV infection of monocytes by antibodies against HIV, *Journal of Biological Physics* 19:133–145

22. Lund O, Hansen J, Sørensen AM, Mosekilde E, Nielsen JO, Hansen J-E S (1995) Increased adhesion as a mechanism of antibody-dependent and anti-body independent complement mediated enhancement of human immunodeficiency virus infection, *Journal of Virology* 69:2393–2400
23. Lund O, Lund OS, Gram G, Nielsen SD, Schønning K, Nielsen JO, Hansen J-E S, Mosekilde E (1997) Gene therapy of T-helper cells in HIV infection: Mathematical model of the criteria for clinical effect, *Bulletin of Mathematical Biology* 59:725–745
24. Mayer G (2010) Microbiology and Immunology Online. School of Medicine, University of South Carolina, <http://pathmicro.med.sc.edu/mayer/phage.htm>
25. Nielsen MH, Pedersen FS, Kjems J (2005) Molecular Strategies to inhibit HIV-1 replication, *Retrovirus* 2:10
26. Nowak MA, Anderson RM, McLean AR, Wolfs TFW, Goudsmit J, May RM (1991) Antigenic diversity threshold and the development of AIDS. *Science* 254:963–969
27. Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, Markowitz M, Ho DD (1997) Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* 6629:188–191
28. Queinnec I, Tarbouriech S, Garcia G, Niculescu S-I (2007) Biology and Control Theory: Current Challenges. *Lecture Notes in Control and Information Sciences* 357
29. Sompayrac LM (2003) *How the Immune System Works*. Blackwell Publishers, Massachusetts
30. Storad CJ (1998) *Inside AIDS: HIV Attacks the Immune System*. Lerner Publications Co. Minneapolis
31. Takeda A, Robinson JE, Ho DD, Debouck C, Haighwood NL, Ennis FA (1992) Distinction of human immunodeficiency virus type 1 neutralization and infection enhancement by human monoclonal antibodies to glycoprotein 120, *J Clin Invest* 89:1952–1957
32. Zucchi R, Ronca-Testoni S (1997) The sarcoplasmic reticulum Ca^{2+} channel/ryanodine receptor: Modulation by endogenous effects, drugs and disease states, *Pharmacological Reviews* 49:1–51

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