Testing HIV Molecular Biology In *In Silico* Physiologies.

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Abstract

The natural and medical sciences have strongly benefitted from technological advances that help to create and store more raw information than can be effectively processed. In particular, this rapid growth has created a strong need for a flexible and far-reaching approach to cross-database simulation. The paper uses a highly simplified example, called the 'TinyMouse' simulator, to explain the design and functioning of interactive cross-database simulators that can be applied to prototype experiments with animal models of human disease, such as the hu-SCID mouse model for the Acquired Immune Deficiency Syndrome (AIDS). Work in progress is discussed to extend 'TinyMouse' into 'CyberMouse', an informational organism that synthesizes factual databases of the murine neuro-endocrine-immune system.

Introduction

The natural and medical sciences have strongly benefitted from technological advances that help to create and store more raw information than can be effectively processed. In particular, this rapid growth has created a strong need for a flexible and far-reaching approach to cross-database simulation.

We have previously described a software thinktank, called the Cellular Device Machine Development System (CDM-DS), for building cross-database simulators with the capacity to predict complex dynamcial behaviors (Sieburg, 1987, 1988, 1990a, 1991a, 1991b). Applications of a CDM-DS derived 'Artificial Immune System' simulator that tests the molecular biology of the Human Immunodeficieny Virus (HIV) in an *in silico* physiological environment have shown that the approach is feasible and can be applied to combat AIDS (Sieburg, 1990b). Undoutedly, because of the generality of the development system, we can address a wider area of human health research that depends on the synthesis of information from multiple sources on multiple levels, in particular the difficult to fathom *in vivo* dynamic linkages between molecular mechanisms and physiologic behaviors. A longterm benefit of using CDM-DS biomedical simulators lies in the reduction of health research costs, support of health policy decisions, and medical education.

Following a brief review of the molecular biology and physiologic impact of HIV in the first section, the second section uses a highly simplified example, called the 'TinyMouse' application (Sieburg, 1993), to explain the design and functioning of a CDM-DS simulator. Although simple, the 'TinyMouse' system can be applied to augment wetlab experiments using mice with severe combined immune deficiency (SCID), which are injected with human peripheral blood lymphocytes (Mosier, 1988, 1991; Torbett, 1991). Sample results from simulations conducted with 'TinyMouse' are described in the third section. The fourth and final section discusses a hypothesis, and mentions work in progress to extend 'TinyMouse' into 'CyberMouse', an informational organism that synthesizes factual databases of the murine neuro-endocrine-immune system.

Molecular Biology and Physiologic Impact of HIV

The Human Immune Deficiency Virus is a complex retrovirus with a known tropism for the cluster designation 4 (CD4) receptor. Infection by the Human Immunodeficiency Virus (HIV) has been shown to induce a large variety of pathological defects in the nervous system (Johnson, 1988; Price, 1988; Grant, 1990), the endocrine system (Dobs, 1988; Biglieri, 1989; Merenich; 1990; Villette, 1990) and the immune system (Fauci, 1988; Rosenberg, 1990; Levy, 1988). The AIDS dementia complex, a progressive neurological syndrome characterized by white and gray matter depletion and abnormalities in cognition, motor performance and behavior, has been indicated as a

common and important cause of morbidity in patients in advanced stages of infection. The critical basis for the immuno-pathogenesis of HIV-infection is the progressive depletion of $CD4^+$ T lymphocytes, a condition eventually resulting in a profound immunosuppression. The understanding of HIV-infection is greatly complicated by the complexity of HIV's target systems (Figure 1).

Another serious factor in the presently unsolved equation of AIDS is that the known HIV strains seem capable of forming a complex system or network of quasi-species in a single host (Meyerhans, 1989), which progressively adapts to the network of biochemical modulators (Castro, 1988; Looney, 1990; Massari, 1990). Therefore, although recent insights into the structure and function of a large array of HIV-1 components have indicated a possible rationale for drug development (Haseltine, 1991; Cullen, 1991), our understanding of the contribution of the structural proteins and regulatory genes in the complex quasi-species life cyles in natural infection is continuously incomplete. Simulation studies of the genetic dynamics of HIV in the context of *in silico* physiological environments appear therefore instrumental to finding a cure to the disease that remains in effect life-long.

The 'TinyMouse' simulator and its extensions in progress, focus on four major strain variations that are amenable to complementary *in silico* and *in vivo* testing: Degree of infectivity, degree of cytopathicity, rate of replication, and sensitivity to neutralization. Variation of infectivity and cell-tropism, cytopathicity, and sensitivity to neutralization, all seem to be related to changes in the *env* gene encoding the gp120 envelope glycoprotein, and the action of late regulatory genes such as *vif* and *vpu*. *Tat* and *rev* appear to be the genes that play a central role in regulating the rate of virus replication.

The suspected physiologic effects on the $CD4^+$ T lymphocyte compartment that can be associated with the above properties classify into 2 major categories, called the intrinsic, and extrinsic pathways, resp. The intrinsic pathway comprises direct killing by cytopathic infection, and physiologic cell death, or apoptosis, a natural suicide mechanism that seems to be triggered by HIV infection (Ameisen, 1992). The extrinsic pathway incorporates the killing of infected cells by cytotoxic T lymphocyes, and pathological cell death by antibody and complement activation. Given the presence of a highly diverse virus population in a single host, it is likely that all these mechanisms are at work, and are triggered at one time or another, or all at once, by the biochemical modulator network, when it is activated by antigen, stress, or other psychosocial factors (Fig. 1). We have previously tested, and confirmed, special cases of this hypothesis in simulation. Specifically, we have shown that frequent immune stimulation, e.g. due to active coinfections, is instrumental in establishing chronic HIV-infection and self-perpetuating cycles of cell activation and bursts of virus-production which subsequently cause a sharp decline in $CD4^+$ T cells at the onset of AIDS (Sieburg, 1990b).

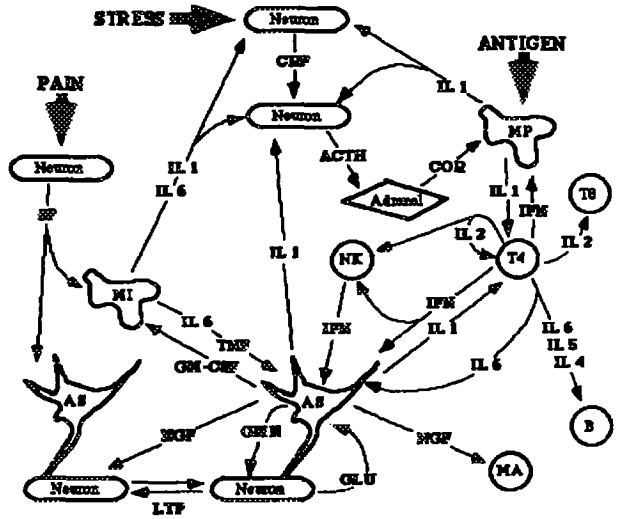

Figure 1. This biomatrix depicts the common molecular language of nervous-endocrine-immune system interactions that are likely to be affected by HIV-infection. Although biologically connected, information about the cell-types and their biochemical connectors are presently only available in isolated factual databases. Pain, stress and antigens can unleash cascades of physiological responses that are mediated and controlled by pleiotropic and pleiotrophic factors. Therefore, in tightly packed tissues such as brain, lymphoid organs, or bone marrow, one secretory product may simultaneously influence the differentiation and proliferation behaviors of several cell-types. During disease, the normal pattern of factor-mediated information-exchange between cells may become distorted and abnormal accumulation or underproduction of their products may result. This can lead to further stress or pain symptoms, or the outbreak of secondary infections. Each in turn may initiate another cascade of physiological responses. It seems therefore likely that an infectious agent, such as HIV, can initiate and subsequently maintain self-sustaining physiological cycles, where modulators

activate productive infection, which in turn activates modulator secretion. It has been proposed that these self-sustaining cycles, or 'phase-lock' dynamics, are characteristic for the physiological pattern-formation that occurs during HIV-infection (Rosenberg, 1990). Our work identifies such patterns by simulation in order to support the development of therapeutic strategies that prevent their formation. We used plain text to indicate cell-types such as 'Neuron' for the neurons of the hypothalamus, pituitary, and hippocampus, 'Adrenal' for the cells of the adrenal cortex, 'AS' for astrocytes, 'MP' for macrophages, 'MI for microglia, 'T4' for $CD4^+$ T cells, 'T8' for $CD8^+$ T cells, 'B' for B cells, 'NK' for natural killer cells, and 'MA' for mast cells. Bold-faced text indicates the cytokines interleukin 1 ('IL 1'), IL 2, IL 4, IL 5, IL 6, interferon ('IFN'), tumor necrosis factor ('TNF'), granulocyte-macrophage colony stimulating factor ('GM-CSF'), the aminoacids glutamate ('GLU') and glutamine ('GMN'), nerve growth factor ('NGF'), corticotropic releasing factor ('CRF'), adrenocorticotropic hormone ('ACTH'), cortisol ('COR'), and the neuropeptide substance P ('SP'). 'LTP' denotes long-term potentiation. Arrows indicate the directionality of signalling between source cell-type and target cell-type. This figure is the basis for extending the 'TinyMouse' simulator into the 'CyberMouse' synthesized database with simulation capacity.

The "TinyMouse" Simulator

The 'TinyMouse' simulator was developed under the CDM-DS version 3 using the SLANG programming language version 3 (Kunzelman, 1993; Grapa, 1993; for a detailled description of version 1, see Sieburg, 1991b). SLANG is an independent language for developing generalized classifier system simulators which are made stand-alone under the CDM-DS. Although the general idea behind our system has not changed from the earlier versions, there are three notable enhancements: First, SLANG is now compiled and not interpreted. Second, we developed the SLANG syntax using a hybrid of C++, BASIC, and PASCAL, so that the language produces self-documenting code which can be run from any text-document (e.g. this paper). Third, the CDM-DS can be run with only minor modifications on any hardware platform, and functions as a shell, much like the C shell or the Bourne shell, under UNIX.

The general philosophy behind a CDM-DS derived simulator is that of a classifier system where pattern elements are manipulated in the context of production rules and fitness functions. It has been shown that such systems are capable of complex computation and learning (Holland, 1986). SLANG, as the first independent programming language for classifier-type simulations, generalizes the notion of classifiers by using object-oriented programming. This allows decentralized manipulation of pattern vectors, called bundles (Sieburg, 1991b), instead of single patterns, the use of object-bound local 'message boards', instead of one global 'message board', and the use of object-specific criteria of fitness. As a true generalization, the system is capable of running a classical classifier system.

Also, under the CDM-DS, a classifier system is given a virtual computer, called a CDM, to run on. This computer is implemented in the form of a 2-dimensional random field, or cellular automaton which, when run asynchronously, allows a classifier to update itself in local neighborhoods relative to a small subset instead of against the whole population of available classifiers. As we have shown in our previous applications, this model of computation greatly fascilitates complex simulation tasks (Sieburg, 1990b, 1991a, 1991b).

The 'TinyMouse' simulator implements only a very small subset of the biomatrix shown in Figure 1. Specifically, its immune system consists of non-interacting T4 cells and macrophages (labelled T4 and MP, respectively, in Figure 1), and only the states 'uninfected' and 'infected' were given to each cell. Cells can be infected by a virus, labelled HIV that is tropic for both the T4 and macrophage cell-types, whenever virus particles are direct neighbors of cells. Also, in radical abstraction from suspected biological reality, infected T4 cells are lyzed immediately and lysis produces a fallout of complete virus particles that can immediately infect uninfected cells. Infected macrophages cannot be lyzed, but instead become 'virus reservoirs' of productive infection producing a fixed number of virus particles at a stochastic rate. The virus population is not affected by the host's cellular or humoral immune responses. Finally, communication between T4 and MP cells is not regulated by biochemical modultors, or cytokines, and the T4 cell compartment is considered a single clone.

Despite the simple physiology of the 'TinyMouse' simulator, we can study an important question that was suggested by the experimental protocols for the SCID mouse model (Mosier, 1988, 1991): What is the dependence of the T4 depletion rate on the dynamic of the unchallenged source of virus? (Figure 2). Also, the simple

system serves as a control for experiments in a larger physiological environment.

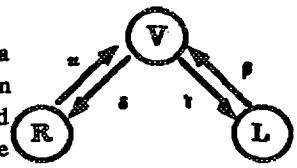

Figure 2. This schema depicts the simulation problem as a directed loss-gain graph of the global dynamics between an unchallenged cellular virus reservoir, labelled 'R', of macrophages, a reservoir of free virus, labelled 'V', and a set of victims, in this case the T4 lymphocytes, labelled 'L'. Since the 'TinyMouse' simulator equates nearest neighbor contact between T cells and virus with cell death, the rate g reflects the actual depletion rate of 'L'. Therefore, if s(R) denotes the size of the reservoir 'R', the question that we are addressing is a functional relationship g = g(s(R)) between the rate g and s(R). The rate b in turn reflects a 'fallout' of complete virus particles from the dying infected lymphocytes. Therefore, as more cells die, b will increase. The rate d reflects the uptake of virus by the macrophages, which then become infected, and this increases s(R). The rate a reflects the secretion of complete virus particles by the reservoir of productively infected macrophages, which thus increases the size of the population 'V' of free virus. One possible extension of the 'TinyMouse' simulator could include a host antibody response. This would pressure the so far unchallenged reservoir 'V', and therefore is expected to help keep the size of 'R' small. In the loss-gain graph, this extension would add one edge, possibly labelled 'B' for antibody-producing B cells, and two vertices reflecting the rates of stimulation of the B cells and their response.

What is the importance of the 'TinyMouse' simulator for wetlab experiments with hu-SCID mice? In this animal model of HIV infection, mice with severe combined immune deficiency, i.e. without lymphocytes, are reconstituted with fixed amounts of human peripheral blood lymphocytes (hu-SCID). After a period of incubation, the reconstituted mice are injected with fixed strains of HIV, each characterized by a fixed set of genetic features. After 2, 4, and 8 weeks, blood is analyzed from the infected mice for the size of the set 'L' (see Figure 2) of human lymphocytes. While these 3 data points show that there is depletion at rates that differ from strain to strain, it is difficult to fathom the relationship between depletion rate and the fluctuating size of the unchallenged reservoir of infected macrophages. Therefore, because the hu-SCID mouse model is a controlled abstraction of the human environment, we can employ an abstracted mouse simulator to obtain intermittant datapoints and thus a more complete profile of the desired functional relationship. Appropriate scaling will establish even a quantitative relationship between *in vivo* and *in silico* population sizes. Before we discuss results in the following section, let us take a look at the 'TinyMouse' code.

The 'TinyMouse' simulator consists of 3 parts: The SLANG script that describes at a high level the interactions between the cell and virus objects (Figure 3), definition files that at a low level translate these interactions into the terminology of classifiers (Figure 4), and a CDM-DS shell script that sets the geometry for the 'TinyMouse' virtual computer, parameters for viewing, initial population sizes, output for time series or spatial data, and the length of an individual run (Figure 5). What is not shown in Figure 5 below because of space constraints, is that the shell scripting language allows us to run - without supervision - a large number of experiments with differing parameters due to the availability of looping.

(Because of further space constraints imposed by the publisher, the author has eliminated Figures 3, 4, and 5 and their legends. The full paper is available upon request to hsieburg@ucsd.edu, or via anonymous ftp to bitmed.ucsd.edu)

Using a build-in preprocessor that operates with file inclusion and '#define' substitution much like the C preprocessor, the high-level interactions implemented in the SLANG script can be reinterpreted into low-level manipulations of bundle classifiers (Figure 4). The 'TinyMouse' simulator uses bundles of length 3. The 'TinyMouse' simulator is run by launching a CDM-DS shell script (Figure 5). In the following section we will describe asample of results that we obtained by running the 'TinyMouse' simulator.

Experiments

To establish a tight correlation between simulation and wetlab experiments, we deviced a scheme for classifying the HIV computer virus strains according to the genetic properties discussed in section 2. The program described in the previous section translates these properties as follows (Table 1): (1) For T cells, cytopathicity equals cytolysis and can thus be measured through direct cell elimination; no cytopathicity for macrophages; (2) Infectivity for both cell-types was set to 'high' by using a 'single hit' approach: Virus close to a cell will be taken up and infects this cell; (3) Replication

of virus occurs in any infected cell and leads to budding of virus (which destroys T4 cells but not macrophages; therefore, macrophages become centers of infection which are not controlled by the 'TinyMouse' immune system); (4) Neutralization was not considered since the 'TinyMouse' immune system does not have B cells.

Table 1. This table depicts the genetics of the V33303300 HIV computer virus. The virus identifier was created as follows: The above table is a matrix of 4 rows and 2 columns which can be written as a vector (v11, v12, v21,..., v41, v42). If we now associate '-'=0 (undetectable or, not tested), '+'=1 (low), '++'=2 (medium), and '+++'=3 (high), then this vector takes the form (3,3,3,0,3,3,0,0). Hence, V33303300. By varying the rates either through user-control or adaptive mechanisms, it is possible to create a cornucopia of other strains. Our short-hand notation helps us to administer the strain families and their genealogy because it is easily interpreted by a computer and can thus be used in a multimedia database system. Use of the latter, e.g. derived from the Macintosh Hypercard system, will allow storing descriptions and kinetics of the real world virus analogs together with data about the computer virus. Conversely, by searching for information about a real world virus, information about the behavior of its *in silico* analog can be extracted including its execution code. We conducted such a comparison of properties which indicates that V33303300 is closer to the HU SF162 and UC1 HIV strains than to the SF2, or SF13 strains.

V33303300	T4	MAC
infectivity	+++	++
cytopathicity	+++	-
replication	+++	+++
neutralization	-	-

To determine the functional relationship discussed in the legend to Figure 2, we conducted 9 experiments using combinations of different initial concentrations of T cells and macrophages. Specifically, we defined three range parameters (L, M, H) for 'low' ($0 \le L \le 33$), 'medium' ($34 \le M \le 66$), and 'high' ($67 \le H \le 100$) percent concentrations. Here, percent concentration is measured according to:

$(\%\ \text{conc})_i$ = ((# cells of type i)/(# cells of all types))*100 .

This absolute measure does not depend on the size of the simulation environment, hence is valid under different configurations of the 'TinyMouse' virtual computer, derives values that can be directly scaled towards the values obtained in the SCID mouse experiments, and allows us to compare simulations of different complexities when we employ additional cell-types (e.g. B cells, and cytotoxic T lymphocytes). The '# cells...' parameter refers to the total number of CDM RAM sites occupied by a specific classifier bundle type. Due to the build-in 'uncertainty' of the simulator, the initial seeding is stochastic, i.e. no two seeding values are precisely the same, thus reflecting the variation typical for wetlab biology. Consequently, a statement like 'was seeded at a low percent concentration' is to be interpreted as 'in the range of low percent concentration'. All experiments were started with a very low dosage of free virus (1 particle), since the unchallenged population of infected macrophages was expected to grow more virus rapidly. Under different settings where this population is challenged by host immunity, we will need to test the depletion dynamic for different infectious dosages of virus.

Using V33303300, each experiment was run for only 31 timesteps (simulation time units), since the experimental conditions imply rapid convergence toward total T4 depletion. In order to detect possible patterns early, we first conducted a 'black box sampling' analysis by extracting cross-sectional datasets at 3 regularly spaced time-points (cmp. Figure 6). Category 20 in Figure 6 singles out 2 datasets, one corresponding to (high T4, low MAC), and the other to (low T4, medium MAC) percent population sizes on startup. Backtracking through the other two categories, we find that for (H,L) there is a slow depletion rate transition from category 10 to category 15, and a fast transition from category 15 to category 20. The (L,M) dynamic is characterized by two consecutive slow transitions. In all other cases, we are looking at consecutive fast transitions.

Interpretation of the (slow,fast) characteristic of (H T4, L MAC) yields that a large T cell population can 'resist' a small population of productively infected cells for an 'extended' period of time, until a threshold is reached beyond which depletion will be very rapid. It is quite possible that, on the microscopic scale of the highly simplified HIV-TinyMouse interaction, we are witnessing the combination of 'latency period' and 'phase transition' that is typical for the clinical onset of AIDS. This conclusion provides a rationale

for extending our experimental system in a controlled way to test the hypothesis that the transition to AIDS requires a combination at the 'right' time of many small dynamics much like the one seen in Figure 6.

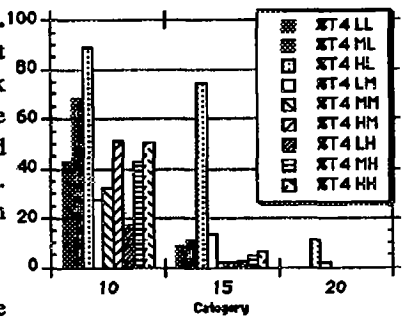

Figure 6. This plot depicts black box data at three regularly spaced time points. Data were taken cross-sectionally over nine experiments on a scale of percent of surviving T4 cells in a population subjected to V33303300.

Although our simulation experiments furbished contiguous time data, the above depiction greatly fascilitates the finding of patterns and also reflects the wetlab situation. A single experiment is uniquely given by the initial concentrations of T4 cells and macrophages that were used to start the system. The use of (L,M,H) is as explained above. In the figure legend, 'LL' means that the experiment was started with a low mean concentration of T4 cells and a low mean concentration of macrophages. All other pairings of L, M, and H are interpreted in the same manner. In biological terms this is equivalent to taking a fluid or tissue sample every x number of hours/days/weeks/... for wetlab analysis. The simulator can therefore be said to serve as a 'black box coprocessor'. The difference between the two systems is that the 'simulation black box' can be lifted for access to the intermittant data and for the 'code' that produced them. Thus the 'black box coprocessor' allows us to back-track from data to mechanisms and thereby to establish a causality chain. It should be mentioned that the 'black box sampling' technique not only gives us a way to run simulations in close association with wetlab evaluation, but is a most effective screening and compression technique when the simulation datasets are very large.

Figure 7 shows the time dynamics for all experiments after lifting the 'black box'. The depletion rate distribution for each experiment can be calculated from these data (not depicted). Since macrophages are not lysed by the virus, their total number remains constant (not depicted).

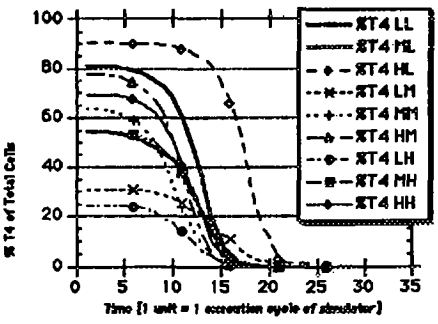

Figure 7. This plot depicts over time the percent of surviving T4 cells in a population subjected to V33303300. Since the experiments only involved CD4+ T cells and macrophages, the "Percent T4" quantity measures the percentage of T4 cells relative to the total number of T4 and macrophages at each time-point (using the formula shown previously). Nine experiments are depicted - 2 representing the 'low' start-up range 0-33%, 3 for the 'medium start-up range 33-66%, and 5 fall into the 'high' start-up range. A single experiment is uniquely given by the initial concentrations of T4 cells and macrophages that were used to start the system. The use of (L,M,H) is as explained above. In the figure legend, 'LL' means that the experiment was started with a low mean concentration of T4 cells and a low mean concentration of macrophages. All other pairings of L, M, and H are interpreted in the same manner. 'LM' and 'HL' are distinguished from the other time-series in that the first shows the slowest overall depletion rate, while the second shows slow depletion first and then, after some critical value is reached, exhibits very fast depletion. The first case indicates that a mid-sized population of productively infected cells yields a slow depletion rate for a small-sized T cell population, and thus may define a 'tail-end phenomenon'. The second case may reflect on a very small scale the 'latency -> AIDS' transition that occurs on a large scale in a whole organism. In fact, the data may show depletion in a small tissue unit, and there may be a way to up-scale the result when a large number of tissue units is combined. One may speculate that in a more realistic set-up where population sizes naturally oscillate, the two critical phenomena can combine and will then show a 'step depletion' that characterizes HIV infection (cmp. 'Discussion' below).

The time series of the population of free virus for all nine experiments also show that the (H,L) and (L,M) dynamics are different from the rest in that the initial concentrations rize slowly before jumping sharply to plateau values of maximal concentration (not depicted). In light of the

previously mentioned hypothesis that explains the macro-dynamcial event 'AIDS' through the accumulation at the 'right time' of many micro-dynamical events, this small-scale observation coinsides with what is seen in natural infection in the human system: low levels of virus titer during the latency period, and high virus titers after the transition to overt disease.

Discussion

The data shown above immediately provide a rationale for extending the experimental system that we worked with so far. Specifically, the next steps should be to (1) introduce some natural fluctuation for the cell populations; this can be done using an object-specific 'eigenlife' fitness function (Sieburg, 1991a, 1991b) together with proliferation capacity; (2) introduce B cells and thus neutralization sensitivity as one of the virus charateristics (Figure 2); (3) implement gradation parameters that control infectivity, cytopathicity, and replication rate. In particular, introducing fluctuation should enable us to investigate the hypothesis that the interaction between some strain of HIV and the 'TinyMouse' artificial immune system can produce a combination of the (H,L) and (L,M) dynamics (Figure 8).

Using a very simple example, we have shown in this paper that cross-database simulation can bridge between isolated information islands that describe biologically connected, yet informationally separated processes. In particular, by immersing information at the molecular level into information at the physiologic level, we made the difficult step forward towards the integration of simulation technology with animal model technology. The next step that is currently in progress in our laboratory, is to expand 'TinyMouse' into the 'CyberMouse' informational organism that integrates the neuro-endocrine-immune biomatrix shown in Figure 1 with other basic facts about the murine system. Undoubtedly, the resulting improved use of existing information across levels will greatly benefit biomedical research.

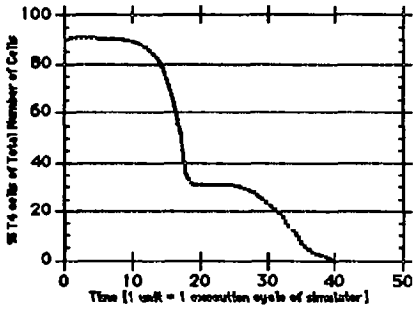

Figure 8. This plot depicts a speculative combination of the (H,L) and (L,M) dynamics shown in Figure 7. Our hypothesis is that this dynamic is achieved in a 'natural' environment when the population of productively infected macrophages expands while the T4 compartment declines. In this case, a critical phenomenon will occur, where the (L,M) dynamic takes control from the previously dominant (H,L) dynamic. Therefore, we would see a dual step pattern of slow depletion followed by rapid depletion that characterizes the transitions from initial infection to the latency period to AIDS. So far, because they are sampled much less frequently than the simulated mice, this phenomenon has not been observed in hu-SCID mice infected with HIV. Thus, further simulations may turn our hypothesis into a prediction that can be easily tested by increasing the sampling frequency *in vivo*. Our finding is therefore an important example for how an *in silico* experimental environment can augment an *in vivo* experimental environment.

Acknowledgements. This work is supported by NIMH grant MH 45688 to HBS. CB and KK acknowledge NSF support through the San Diego Supercomputer Center "Research Experience for Undergraduates" program. The authors are grateful for suggestions and critisism by Drs. Donald E. Mosier and Christa E. Müller-Sieburg. Finally, the authors, and Michele Dafoe as another member of the team, would like to congratulate themselves for having done the publishers work of type-setting this paper. In the future, we would, however, prefer to spend our time, and the Public Health Service's financial support, on our scientific goals, and not on a career in type-setting.

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