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# ABSTRACT

**Title of Thesis :** Mathematical Modeling and Simulation of the Progression of AIDS

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**Thesis Directed by :** Sam S. Sofer, Professor  
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Acquired Immunodeficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV) is a pernicious disease acknowledged as a world health problem. One of the major issues in the study of AIDS is understanding the manifestation and the progression of the disease following the transmission of HIV. Unfortunately, the present state of biological knowledge does not yet give us a reliable means of evaluating these phenomena. A simulation approach is, therefore, extremely useful in predicting the various phases of the deteriorating physiological status of the patient. In the present study we have focused on the development of a mathematical model of the immune response to HIV and subsequently the dynamics of AIDS progression. The model is based on a set of ordinary differential equations solved using numerical analysis techniques. The model predicts the response of the T4 helper cells, cytotoxic and natural killer cells, macrophages and monocytes, and antibodies to the infection. Three cases are presented in this thesis. Each case has a different initial concentration of the virus. The rate of depletion of the immune cells is directly proportional to the increase in the initial concentration of the virus. These model-based investigations show that the theoretical results generated by the model are in close agreement with real life clinical observations of patients.

A hypothetical detoxification unit is proposed for the treatment of AIDS. It is believed that this detox unit may not be able to cure the patient, but would at least increase the patient's life. Simulation results of AIDS progression using this detox machine are plotted in this thesis. The detox unit is in the experimental stage of blood separation. The experimental procedures and results obtained from the detox machine up to this day are also presented in the following chapters. Further research in this field is in progress.

## KEYWORDS:

SIMULATION      AIDS      HIV INFECTION  
T4 HELPER CELLS      ANTIGEN

2) **MATHEMATICAL MODELING AND SIMULATION  
OF THE PROGRESSION OF AIDS**

1) **BY  
ANJU H. NAGPAL**

**Thesis submitted to the faculty of the Graduate School of New Jersey Institute of  
Technology in partial fulfillment of the requirements for the degree of  
Master of Science in Biomedical Engineering.  
1990.**

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# CHAPTER 1

## INTRODUCTION

The immune system is a very complex network of white blood cells, proteins and cofactors. It is mainly responsible for the protection of the body against foreign organisms. Individual patients exhibit unique immune system profiles. Major parameters that affect the individual's system response to the attacker include concentration levels of white blood cells (WBC) and immunoglobulins (Ig), and the distribution of the WBC subpopulations and immunoglobulins [1,2]. In characterizing the immune state it is also necessary to consider the day-to-day and patient-to-patient variations in these physiological and biochemical functions.

Mathematical modeling capable of incorporating these parameters and therefore accurately reflecting their influence on the system provides a powerful tool for predicting the immune response.

In studies related to Acquired Immunodeficiency Syndrome (AIDS), such models for simulating immune response are expected to provide information that may be critical in understanding the etiology of the disease. The search for treatment, or at least longer survival for an AIDS patient has gained increased momentum in recent years [3]. Current research has focused on the design and testing of several anti-viral drugs for AIDS. With the growing need for treatment of this fatal disease, simulation of the immune system can help us test and evaluate the effect of different drugs (presently available as well as future possible solutions evolving from research) on the patient.

In this thesis we have developed a mathematical model of the immune response to HIV in order to understand the dynamics of AIDS progression. The model consists of a set of nonlinear, first order differential equations. The model predicts the response of T4 helper cells, monocytes and macrophages, cytotoxic and natural killer cells, and antibodies to the antigen. The procedure for testing the progression of the disease is based on assumptions of initial concentration of HIV virus present in blood at the moment of transmission. We have considered three cases of AIDS progression in which the initial concentration of the virus is varied from 10 to 100 virus particles/ $\mu$ l of peripheral blood. These cases are simulated again using the detoxification unit.

# CHAPTER 2

## PHYSIOLOGY OF THE IMMUNE SYSTEM

The body, a multicellular organism, may be thought of as an ecosystem containing numerous niches that can be occupied by organisms uniquely adapted to the prevailing environment. One such niche in this ecosystem is the cell, which can be invaded by foreign disease-producing organisms.

The immune system is made up of a complicated network of white cells and chemical products which interact synergistically to eliminate foreign invaders, abnormal cells, and toxic cell products (Figure 2.1).

Basically there are have two special types of white cells; monocytes and macrophages, and the lymphocytes.

The white cells originate from the stem cells found in the fetal liver and the bone marrow. They differentiate into many cell types as they mature and these cell types may have separate or overlapping functions. Most white cells in the blood are short-lived scavenger cells (neutrophils) that engulf and digest foreign microbes and die.

The reticulo-endothelial system comprises monocytes and macrophages. The monocytes and the macrophages are also scavenger cells, but they have a longer lifetime, perhaps months or years. They are particularly good at detecting, engulfing, and digesting tumors or virally infected cells. Some monocytes circulate in the bloodstream and later in life receive immune signals from the lymphocytes that cause the monocytes to migrate into tissues and transform into tissue specific macrophages. From that point they follow two pathways; either they wander freely through the connective tissue in organs or attach to the basement membrane of the tiny capillaries in those organs. Most of the monocytes differentiate directly into tissue specific macrophages. The macrophages are concentrated and strategically located in organs like the liver and lungs, and in the lymph nodes. These tissues receive blood from the parts of the body that are exposed to the outside world. If the invading organism escapes this early level of immune defense, then they reach a second level of defense which is provided by the spleen, kidney, joints, and brain. This system is one major line of defense.

Another major line of defense in the immune system is the lymphoid system, a set of glands, organs, and cells. The T and B cells are manufactured and shipped from the lymph nodes that are situated throughout the body. In the process of maturing the lymphocytes differentiate into hundreds of thousands of lymphocyte subgroups, each very small and each designed to recognize and mount a defense against a specific foreign protein or antigen.

### Basic Functions of the immune system

Immunology is concerned with cells and molecules spread throughout the body, the functional connections between which have been apparent for at least 40 years.

The task confronting the natural defense system can be capsulized in six key words:

Encounter, recognition, activation, deployment, discrimination and regulation.

Before the immune response is invoked, the invading microbe and antigens derived from it must encounter cells capable of responding.

There are several kinds of antigens and their entry into the body depends on the category or class of antigen to which they belong.

Precise and elaborate mechanisms target antigens to sites where the opportunity for encounter with lymphocytes is optimized. Obviously since immunity is specific, molecular mechanisms of recognition must exist. Both the antigen-specific receptors on lymphocytes and the antibody molecules that are eventually formed must recognize and unite with particular antigenic molecules or molecular fragments. It is impossible for the immune system to know in advance which specific type of antigen it may be asked to recognize. With the invasion of the antigen the system must somehow possess the capability of responding to it.

After recognition the lymphocytes must be activated to respond, either by producing antibodies or by acting in the inflammatory response. The antibodies or the lymphocytes alone cannot rid the body of the invader. Instead, they cooperate in an elaborate deployment strategy, amplifying and distributing the defense function and collaborating their attack with other non-antigen-specific cells (such as macrophages, phagocytes) or molecules (such as the complement system) in destroying and eliminating the pathogen.

The immune system has the ability to discriminate between self and non-self, thereby avoiding auto-immune tissue damage.

The immune system tolerates many autologous antigens. The positive and negative feedback loops control the different components of the immune system and maintain them within the permissible range, thus taking care of immune regulation.

Macrophages entering the lymph nodes or interacting with lymphocytes in tissues have the job of presenting foreign antigen to the appropriate lymphocyte subgroup and thereafter activate it. The process involves engulfing and digesting foreign cells and infected cells, incorporating into their surface the proteins of the foreign invaders. The foreign antigens are inserted on the macrophage surface next to other normal receptors, called MHC antigens (antigens of the major histocompatibility complex). The MHC antigens are part of the associative recognition network of surface receptors that enable the macrophages and lymphocytes to recognize each other as parts of self and to receive appropriate instructions from each other. When a foreign antigen is present on the macrophage surface, only those lymphocytes that recognize or bind to both an MHC receptor and the specific foreign antigen are activated.

## **IMMUNOLOGY OF AIDS**

Helper/inducer T lymphocytes bearing CD4 protein molecules have been termed as “the leader of the immunological orchestra” because of their central role in the immune response [4,5,6,7,8] (Figure 2.2).

Many immune responses are induced by dual recognition between a T4 helper cell and an antigen presented by macrophage. Contact between T4 cell receptor, the MHC (major histocompatibility complex), and the foreign antigens of the macrophage stimulates the T4 lymphocytes to send out chemical instructions to other immune cells. The chemical instructions in the form of lymphokines, induce a variety of effects: they activate monocytes and macrophages, thereby enhancing their ability to engulf and destroy the invading pathogen; they stimulate cytotoxic lymphocytes, called T8 killer cells, to proliferate and kill infected cells. They also stimulate B lymphocytes to proliferate and produce antigen specific antibodies capable of binding to the foreign antigen [1].

The main target of the virus is the helper/inducer cells which is a subset of Thymus derived (T) lymphocytes. HIV infects T4 cells by binding to the CD4 protein receptor present in great abundance on the surface of these cells [4,5,6,7,8,9,10]. Some monocytes and macrophages, and some B cells also carry CD4 molecules on their surface [5,6]. When HIV attacks a T4 helper cell, its envelope glycoprotein (gp120) binds to CD4 [4,5,6,7]. The stem (transmembrane protein gp41) attached to gp120 then inserts itself into the cell membrane; the viral and cell membranes fuse together; and the virus dumps its genetic contents into the interior of the cell [5,6,7] (Figure 2.3). Thus, by taking advantage of the recognition network in the immune

system, the virus gains entry into cells that are attempting to fight off foreign invaders. The destruction of CD4 bearing lymphocytes probably accounts for the immunosuppressive effect of the virus.

The normal function of the T cells is thus impaired and the ability of T cells to recognize foreign substances and stimulate B lymphocytes is lost. As a result B cells are unable to multiply and produce specific antibodies that destroy the invading virus [7]. In addition, infected helper T cells serve as hosts where the virus flourishes until the host cells rupture and introduce large amounts of virus into the blood stream [6] (Figure 2.4).

The progressive decline in the number of T4 cells is correlated with the progression of AIDS from the initial infection stage to the terminal stage [4 - 8,11].

Individuals may remain seropositive and healthy for long periods but a hallmark of disease progression is an inexorable fall in the number of helper/inducer CD4 positive cells [7,11]. In full blown AIDS there is also a fall in the number of CD8 lymphocytes [4,7]. The monocyte/macrophage function, including the ability to kill intracellular parasites, is impaired [9]. Natural killer cell activity is also decreased [9,10].

The simplest hypothesis for the cause of immune deficit is the destruction of helper/inducer T cells and probably also monocytes and macrophages by the virus [5,6]. Another possibility is that the envelope glycoprotein of the virus which binds to CD4, may interfere with the normal function of this molecule. Since CD4 plays a part in the interaction of helper/inducer T cells with other cell types it would block their normal function and result in immune dysfunction [4].

Fig 2-1

### WHITE CELLS OF THE IMMUNE SYSTEM

(a) GENESIS OF WHITE CELLS

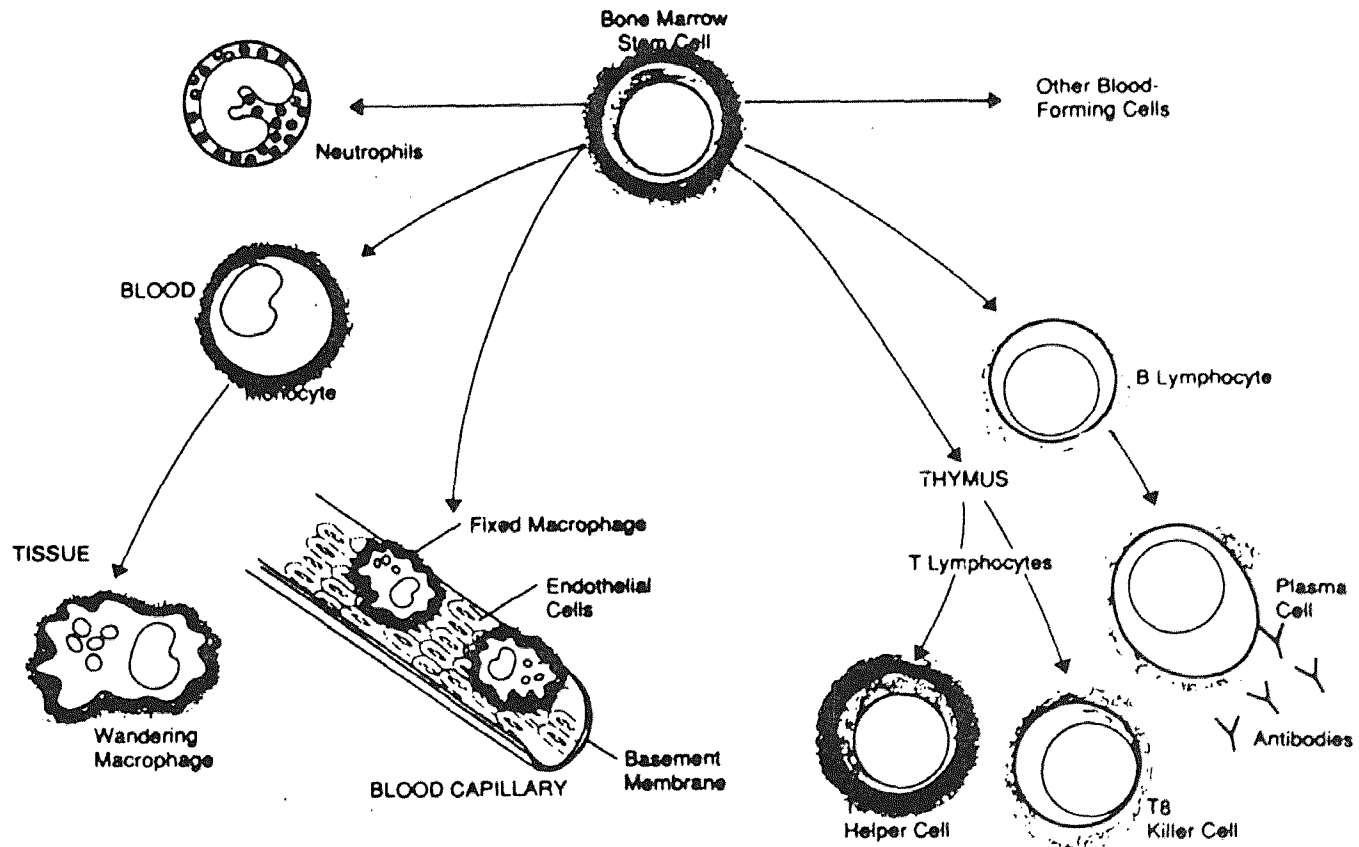
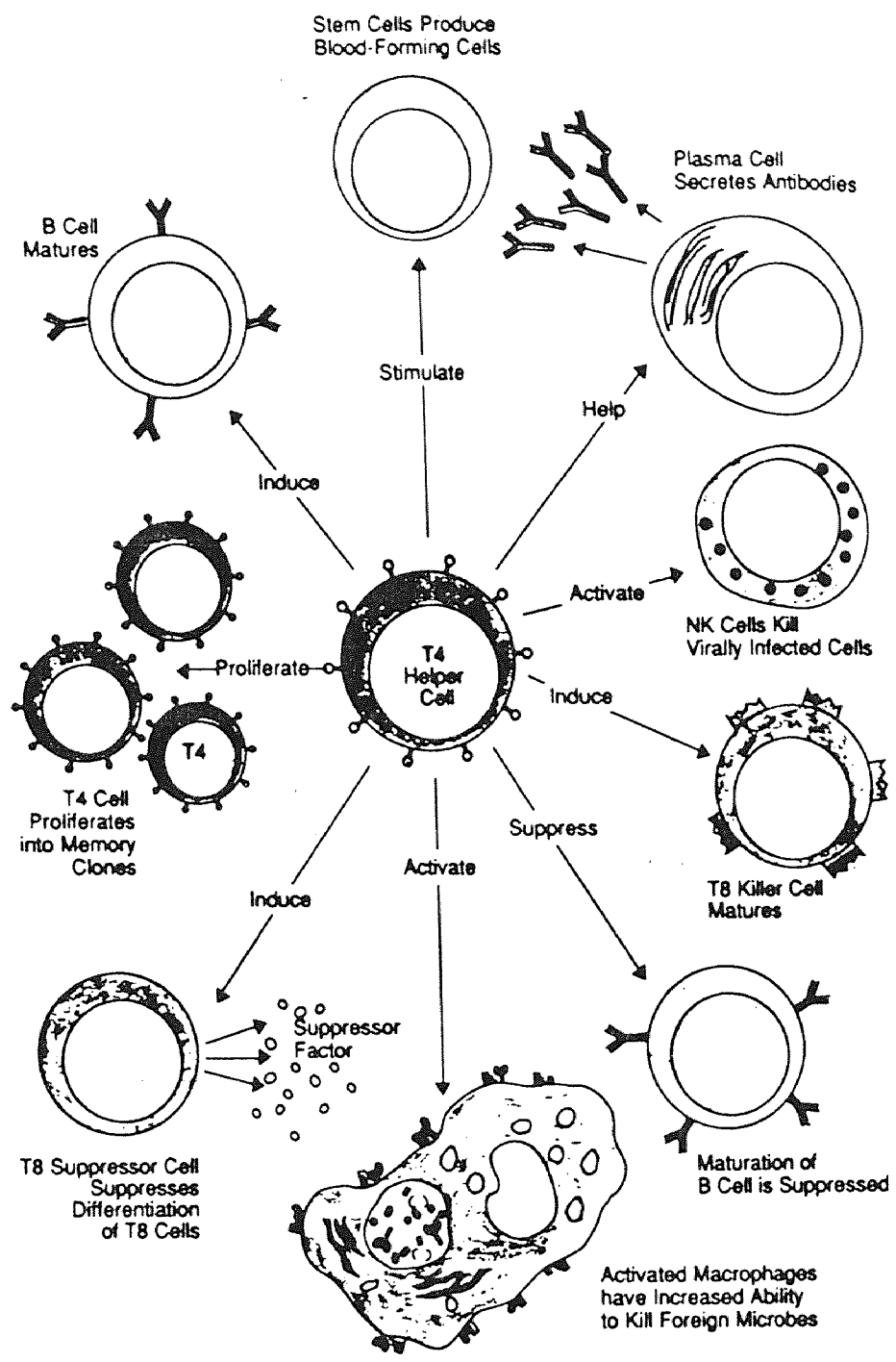


Fig 2.2

# FUNCTION OF T HELPER CELLS

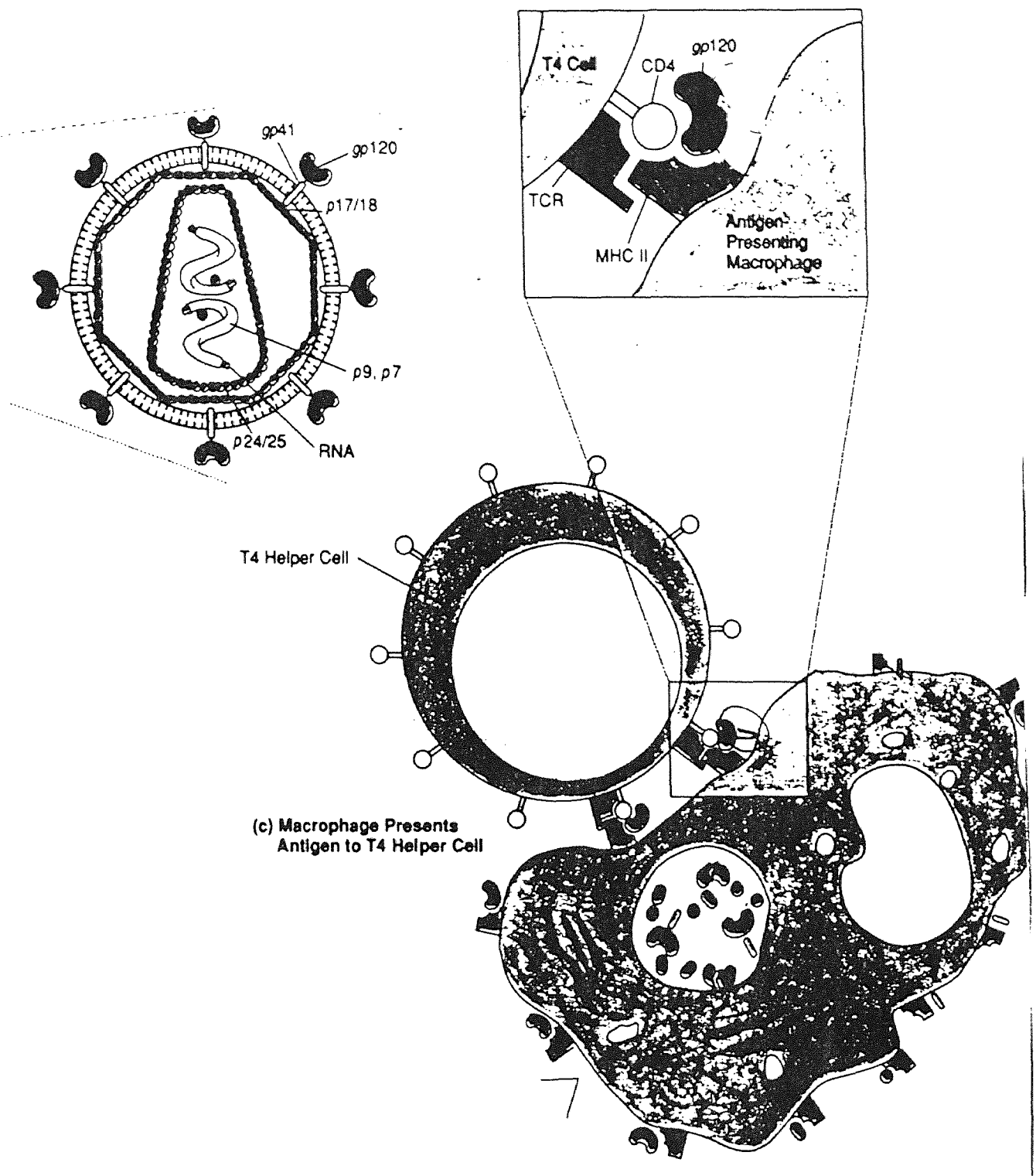


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Fig 2 B

# HIV ATTACKS T-HELPER CELLS



# INFECTION OF CELLS

## STAGE 1 HIV through Cell Fusion Transmission

1. Extracellular signals or normal cell contacts activate infected macrophage.

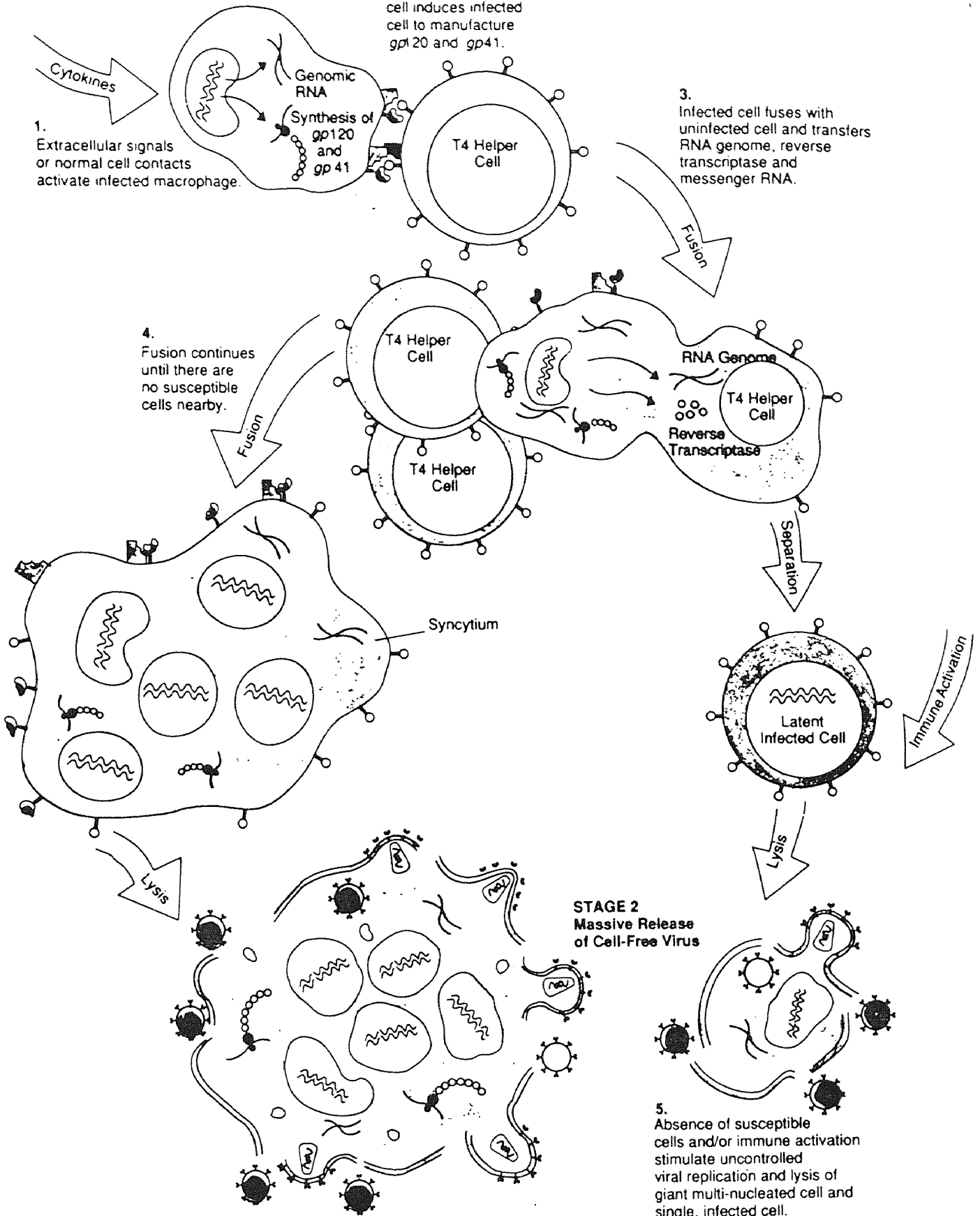
2. Contact with nearby cell induces infected cell to manufacture gp120 and gp41.

3. Infected cell fuses with uninfected cell and transfers RNA genome, reverse transcriptase and messenger RNA.

4. Fusion continues until there are no susceptible cells nearby.

## STAGE 2 Massive Release of Cell-Free Virus

5. Absence of susceptible cells and/or immune activation stimulate uncontrolled viral replication and lysis of giant multi-nucleated cell and single, infected cell.



# CHAPTER 3

## MODEL OF THE IMMUNE SYSTEM

The mathematical model presented here is developed using the concept of single compartmental analysis [12 - 14]. The central aspect incorporated in the model is the multiplication and destruction of white blood cell populations. Destruction, either by cell malfunction after combining with the antigen or normal cell aging, is possible.

The virus multiplies at a certain rate in the patient [5 - 7,12,13]. This rate is proportional to the number of infected T cells because these cells provide a niche for the virus. Within the infected T cells the virus can stay transient for a certain period but when the virus gets activated it multiplies at a very high rate. The cells burst and the virus spreads in the blood stream [5,6]. T cells, the primary proponents of the defense system, are unable to produce stimulating chemicals for the immune components to respond against the virus, hence the entire immune system completely breaks down.

If appropriate measures are taken in the initial stage, there can be an elimination or at least reduction in the number of infected cells, thus resulting in a decrease of the virus concentration.

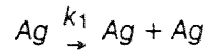
One proposed cure which is presently in the research stage is processing the blood in an extracorporeal detoxification system (detox unit). In the detox unit the virus and infected WBCs are removed by selective sedimentation and by the anti-viral device. It is assumed that viral infection changes sedimentation characteristics of the infected WBCs (a fact demonstrated in mouse cells by Welsh ) [9-12]. The detox unit is based on the principle that the infected white blood cells have different sedimentation properties compared to the normal white blood cells, therefore it is possible to separate them.

There are chances of the cells breaking due to physical agitation and fluid shear stress. That would rupture the infected WBCs pouring the virus content into the blood stream and also damaging a few healthy cells.

We have assumed that viral reproduction takes place by infection as well as being a direct function of free virus concentration [12,13]. Virus multiplies within the cell [5,6,7]. The virus can

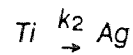
stay quiescent for an unpredictable duration. We have assumed that there is constant multiplication of virus [12,13].

From the above discussion the following relations can be written down:



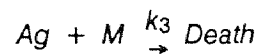
Antigen yields antigen

(1)



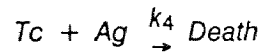
Infected cells yield antigen

(2)



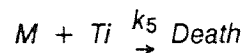
We have assumed the destruction of the antigen and monocytes or macrophages after binding together. These should be extended to include the cases where antigen is destroyed without killing the monocytes or the macrophages.

(3)



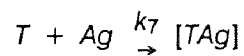
We have assumed the destruction of the cytotoxic cells and antigen after binding together. This term should also be extended to include cases where antigen is destroyed without killing the cytotoxic or natural killer cells.

(4)



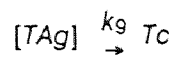
Death refers to destruction of monocytes or macrophages and the infected cells.

(5)



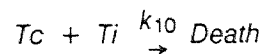
Antigen and T4 cells bind together to form a complex.

(6)



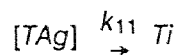
Production of cytotoxic cells by the stimulus of the [TAg] complex

(7)



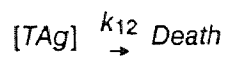
Cytotoxic cells attack the infected T cells, resulting in the destruction of both the cells.

(8)



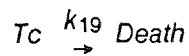
Formation of infected T cells from the [TAg] complex.

(9)



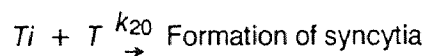
Destruction of the complex in the body due to high concentration of antigen.

(10)

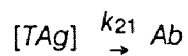


Destruction of cytotoxic and NK cells by infected T cells.

(11)

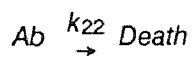


(12)

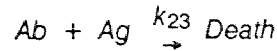


Antibody production is stimulated by T-Ag complex

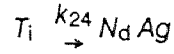
(13)



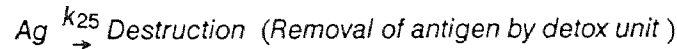
Antibodies are destroyed by infected T cells



Antibody binds with antigen resulting in destruction of both the molecules.



Infected cells break due to detox unit and release antigen



(14)

(15)

(16)

(17)

## System Equations

Combining the above equations, the overall system of differential equations can be described as :

### Change in Antigen concentration with respect to time:

The antigen concentration is directly proportional to free antigen concentration [Ag] and the number of infected T cells [Ti]. The antigen concentration decreases when immune cells (monocytes [M], T4 cells [T], cytotoxic cells [Tc] and antibodies [Ab]) combine with the antigen. When the detox machine is turned on some antigen may be released in the blood stream due to the breakage of the infected cells (Nd[Ag]) and some would be removed by the detox unit.

$$\frac{d[Ag]}{dt} = k_1 [Ag] + k_2 [Ti] - k_3 [M][Ag] - k_4 [Tc][Ag] - k_7 [T][Ag] - k_{23} [Ab][Ag] + N_d k_{24} [Ag] - k_{25} [Ag]$$

(18)

### Change in T4 helper cell concentration with respect to time:

The T4 cell concentration is proportional to the production of T4 cells ( $k_6/(k_{13} + [T])$ ). The concentration decreases when T4 cells combine with the antigen [T][Ag] to form antigen-T cell complex [TAg]. Their concentration also decreases when the T4 cells combine with the infected cells ([T][Ti]), thus forming syncytia. The concentration of T4 cells is proportional to the destruction of T4 cells ( $k_{14} * T / (k_{16} + T)$ ) due to aging.

$$\frac{d[T]}{dt} = \frac{k_6}{k_{13} + [T]} - k_7 [T][Ag] - \frac{k_{14} [T]}{k_{16} + [T]} - k_{20} [T][Ti]$$

(19)

Change in other CD4 cells (monocytes and macrophages) concentration with respect to time:

The concentration of CD4 cells other than T4 cells increases with their production rate ( $k_8/(k_{15} + M)$ ) and decreases with their destruction rate ( $k_{17}M/(k_{18} + M)$ ). The concentration of these cells may also decrease when these cells combine with antigen ( $[Ag][M]$ ) or infected T cells ( $[M][Ti]$ ).

$$\frac{d[M]}{dt} = \frac{k_8}{k_{15} + [M]} - k_3 [Ag] [M] - k_5 [M][Ti] - \frac{k_{17} [M]}{k_{18} + [M]}$$

(20)

Change in Cytotoxic and natural killer cells concentration with respect to time:

The concentration of cytotoxic and natural killer cells is directly proportional to the antigen-T cell complex  $[TAg]$ . This complex stimulates the production of cytotoxic cells which develop from mature T cells. Their concentration decreases when the cells attack the antigen ( $[Tc][Ag]$ ) and the infected cells ( $[Tc][Ti]$ ). The concentration also decreases as the number of infected cells  $[Ti]$  increases.

$$\frac{d[Tc]}{dt} = k_9 [TAg] - k_4 [Tc][Ag] - k_{10} [Tc][Ti] - k_{19} [Ti]$$

(21)

Change in Infected cell concentration with respect to time:

The concentration of infected cells increases when the T cell-antigen complex  $[TAg]$  results in infected T cells. The infected T cells also combine with healthy T cells ( $[T][Ti]$ ), thus forming syncytia which further increases their concentration. The infected T cells concentration decreases when the cytotoxic cells, NK cells ( $[Tc][Ti]$ ), monocytes or macrophages ( $[M][Ti]$ ) recognize the infected cell and try to destroy it. When the detox unit is turned on some infected T cells may be removed and some may break.

$$\frac{d[Ti]}{dt} = k_{11} [TAg] - k_5 [M][Ti] - k_{10} [Tc][Ti] + k_{20} [T][Ti] - k_{24}[Ti]$$

(22)

Change in T cell and antigen complex with respect to time:

The complex concentration increases proportionately to the concentration of antigen as well as T4 cells ( $[T][Ag]$ ). Their concentration decreases if the complex  $[TAg]$  transforms into an infected T cell  $[Ti]$  or the complex is destroyed due to high concentrations of the antigen  $[Ag]$ .

$$\frac{d[TAg]}{dt} = k_7 [T][Ag] - k_{11} [TAg] - k_{12} [Ag]$$

(23)

Change in Antibody concentration with respect to time:

The antibody production gets stimulated by the T cell-antigen complex  $[TAG]$  and the concentration decreases proportionately to the number of infected T cells  $[Ti]$ . Their concentration also decreases with the combining of antibody to the antigen  $[Ab][Ag]$ .

$$\frac{d[Ab]}{dt} = k_{21} [TAG] - k_{22} [Ti] - k_{23} [Ag][Ab]$$

(24)

where :

- Ag antigen concentration per  $\mu$ l
- T T4 helper cell concentration per  $\mu$ l
- M other CD4 cells (monocytes and macrophages)
- Tc cytotoxic and natural killer cells concentration per  $\mu$ l
- Ti infected T4 cell concentration per  $\mu$ l
- TAg concentration of T4 cell and antigen complex per  $\mu$ l
- Ab antibody concentration per  $\mu$ l
- $k_1$  rate constant for antigen multiplication
- $k_2$  rate constant for antigen replication within an infected T4 cell
- $k_3$  rate constant for destruction of other CD4 cells and antigen after binding.
- $k_4$  rate constant for destruction of cytotoxic and NK cells , and antigen after binding
- $k_5$  rate constant for destruction of other CD4 cells and infected T4 cells after binding
- $k_6$  rate constant for normal production of T4 cells
- $k_7$  rate constant for formation of T4 cells and antigen complex
- $k_8$  rate constant for normal production of other CD4 cells
- $k_9$  rate constant for production of cytotoxic cells stimulated by TAg complex
- $k_{10}$  rate constant for destruction of infected cells and cytotoxic cells after binding
- $k_{11}$  rate constant for formation of infected T cells
- $k_{12}$  rate constant for destruction of TAg complex
- $k_{13}$  rate constant for regulation of T4 cell production
- $k_{14}$  rate constant for normal destruction of T4 cells
- $k_{15}$  rate constant for regulation of other CD4 cells production
- $k_{16}$  rate constant for regulation of T4 cell destruction



- $k_{17}$  rate constant for normal destruction of other CD4 cells
- $k_{18}$  rate constant for regulation of other CD4 cells destruction
- $k_{19}$  rate constant for destruction of cytotoxic and NK cells due to infected T4 cells
- $k_{20}$  rate constant for formation of syncytia
- $k_{21}$  rate constant for production of antibodies
- $k_{22}$  rate constant for destruction of antibodies due to infected cells
- $k_{23}$  rate constant for destruction of antigen and antibody after combining
- $k_{24}$  rate constant for the breakage as well as removal of infected cells and release of Ag
- $k_{25}$  rate constant for the removal of antigen by the detox unit
- $N_d$  average number of virus from breakage of infected cells by detox unit

# CHAPTER 4

## ESTIMATION OF MODEL PARAMETERS

Clinical data were available for the concentration of T4 helper cells, monocytes and polymorphs in healthy individuals. The normal count of T4 cells is  $1000/\mu\text{l}$  and the combined normal count of monocytes and polymorphs is  $5000/\mu\text{l}$  [15]. The concentration of cytotoxic cells and antibodies to the virus are zero for an individual not infected by AIDS. Assuming the concentration of antigen to be zero, the production and destruction rate constants for differential WBCs are calculated.

Using engineering and physiological judgment, the equations have been formulated so that the rate of production is equal to the rate of destruction of the cells at homeostasis. The cell reproduction rate is not a direct function of cell type, however, a high count of differential cells is an indication of specific type cell reproduction ability [12,13]. The production and destruction rate of the differential WBCs may vary from patient to patient depending on their health. For this model we have assumed a value and kept it constant in all the case studies.

### STEP 1:

This is a simple case where no AIDS virus is injected in the body. It is the starting case for the simulation model. It has been shown that with no introduction of the antigen, the differential components of the white cells remain unchanged. When no HIV antigen is present in the human body, a healthy state is maintained by the individual. This condition is applicable to a healthy person whose differential WBC count is always maintained normally. The results obtained from the simulation of this step is shown in Figure 1. This model is rigid with absolute numbers but in reality, concentration of cells in the body may vary slightly above or below the rigid line.

### STEP 2:

The next step in deriving the parameters is to simulate a case of perturbation and recovery to homeostasis. In this case the initial parameter of any component of the immune system is shifted from the normal concentration. After a certain period of time, the cells whose concentration was shifted come back to their normal required concentration within the body. In the model this period can be varied by increasing or decreasing the rate constants responsible for production or destruction of the respective cells. For example, we can shift the initial

concentration of T cells to 500 /ul which is much less than the normal value (Figure 2). At first the rate of change of T cells is high in order to get the T cell concentration closer to the normal range. The rate of change of T cells is dependent on the rate constants  $K_6$ ,  $K_{13}$ ,  $K_{14}$ ,  $K_{16}$ .

Increasing  $K_6$  increases the concentration level due to increase in the production rate. Decreasing  $K_{14}$  also increases the concentration of T cells since it corresponds to the destruction rate of T cells. When the concentration is decreased below 1000 /ul,  $K_{13}$  works effectively in bringing the concentration back to normal by creating an additive effect in the denominator value. The denominator value is decreased due to which reproduction rate increases.  $K_{13}$  is primarily needed to prevent an abnormal positive spike of T cells. It is necessary that the rise in T cells be gradual.

Rate constants  $K_{14}$  and  $K_{16}$  work similarly. For a healthy person T4 cell concentration is equal to the value of the rate constant  $K_{16}$ . When the T cell concentration decreases the denominator value decreases proportionately and the numerator value also decreases considerably because  $K_{14}$  is a direct multiple of T4 cell concentration. The overall result is decrease in the destruction rate.  $K_{13}$  and  $K_{16}$  are the rate constants for the regulation of T cells in order to maintain their level in the normal range. Thus when there is a decrease in the T4 cell concentration, reproduction rate is increased and the destruction rate is decreased, the overall effect being an increase in the concentration of T4 helper cells back to normal.

Thus, if the normal cell count of T4 cells or polymorphs were decreased due to some infection other than HIV, the immune cells reach normal levels after a certain period of time as shown in Figure 2. This case also proves that other infections do not destroy the immune structure completely and that the individual can recover. The duration of recuperation depends on the production and destruction rate constants. Table 1 shows the initial values of the variables parameters used in the model.

Table 1

Initial Concentrations:		
Case 1	Case 2	Case 3
Antigen = 10.0/ $\mu$ l	Antigen = 50.0/ $\mu$ l	Antigen = 100.0/ $\mu$ l
Initial Conditions		Rate Constants
T4 cells = 1000	k <sub>1</sub> = 0.0000001	k <sub>13</sub> = 50.0
other CD4 cells = 5000	k <sub>2</sub> = 1.2	k <sub>14</sub> = 1.8
cytotoxic, NK = 0.0	k <sub>3</sub> = 0.000001	k <sub>15</sub> = 50.0
infected T cells = 0.0	k <sub>4</sub> = 0.0001	k <sub>16</sub> = 1000.0
T-Ag complex = 0.0	k <sub>5</sub> = 0.000000001	k <sub>17</sub> = 4.0
Antibodies = 0.0	k <sub>6</sub> = 1000.0	k <sub>18</sub> = 5000.0
	k <sub>7</sub> = 0.0009	k <sub>19</sub> = 0.022
	k <sub>8</sub> = 10100.0	k <sub>20</sub> = 0.00001
	k <sub>9</sub> = 0.00004	k <sub>21</sub> = 0.0001
	k <sub>10</sub> = 0.0000001	k <sub>22</sub> = 0.09
	k <sub>11</sub> = 0.000001	k <sub>23</sub> = 0.00001
	k <sub>12</sub> = 0.00041	

By studying the reaction of antigen binding to the different immune cells, we assumed that each combination is proportional to the concentration of the two cells involved. The rate at which the antigen combines with the differential WBC cells is assumed with the knowledge of their functional behavior in response to the virus.

This is a patient customized model. Periodically WBC counts of the patient should be taken. The change in the condition of the patient's health should be perused for a few days to provide patient specific values for the constants in the model. Deterioration of the patient's health should also be used when calculating and verifying rate constants in the above equations.

The patient specific model should then be simulated, thus detecting tolerance to the virus. Response to the infection can be studied by plotting a graph of differential WBC components versus time. Once the immune system is tracked, different approaches can be simulated for the patient's treatment. Diverse drugs can be tested using the model and the results obtained from simulation can help the doctor in selecting the best treatment for that patient.

# HOMEOSTASIS CONDITION

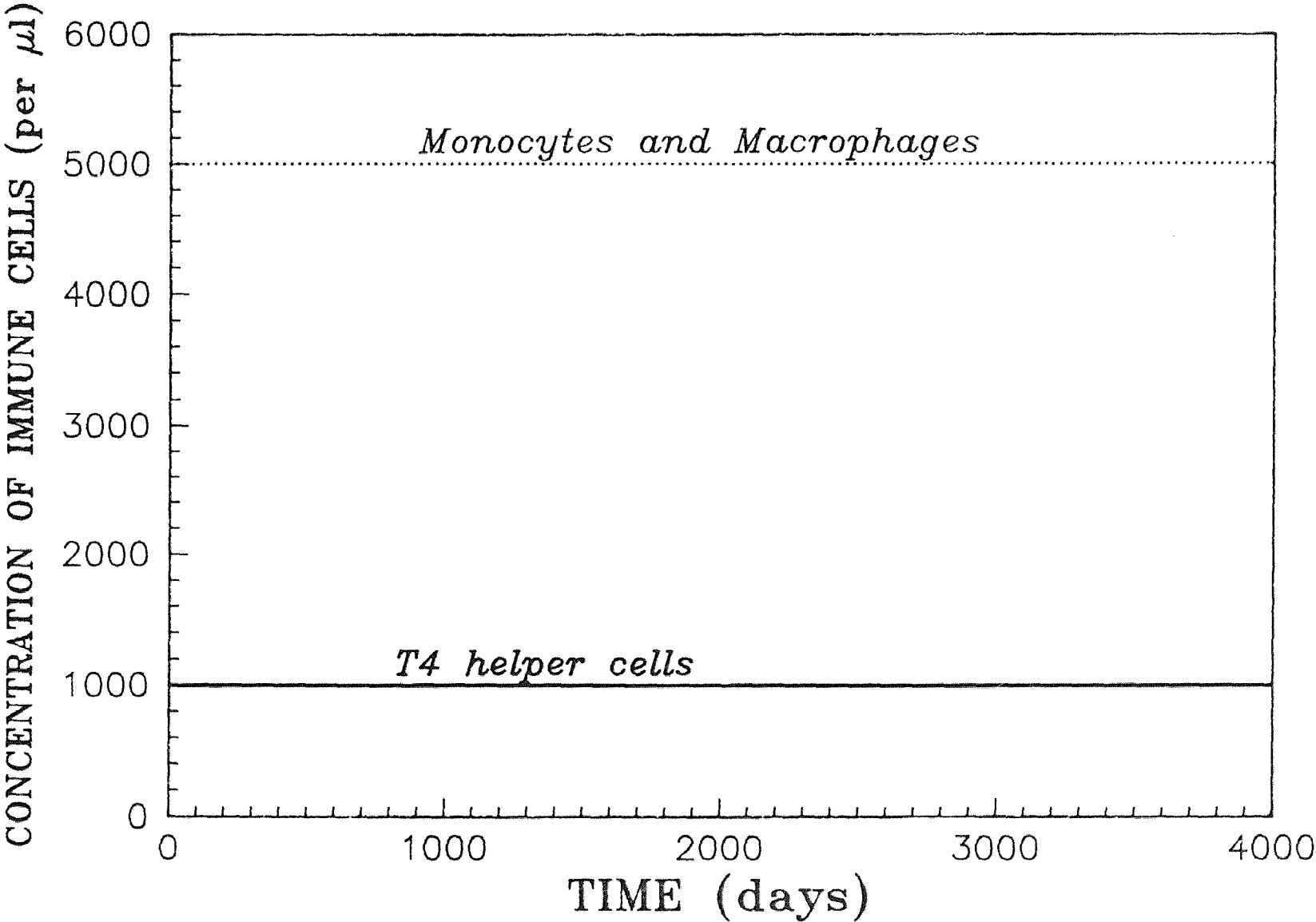


Figure 4.1

# RECOVERY TO HOMEOSTASIS

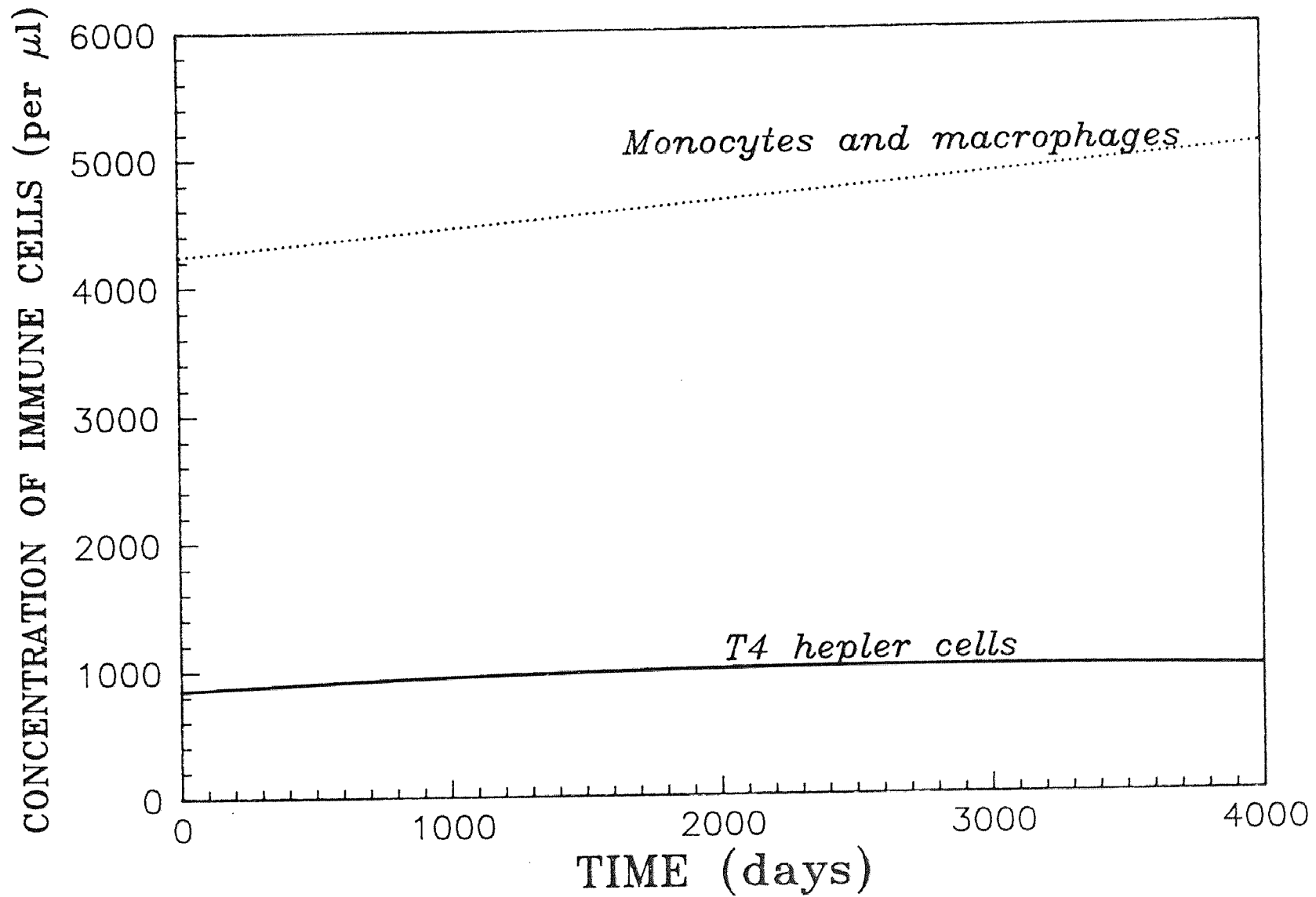


Figure 4.2

# CHAPTER 5

## **Experimental work performed using extracorporeal detoxification unit**

The primary objective of the experimental work was to attain optimization of white blood cell separation.

This chapter deals with the experimental techniques and features of the detoxification unit [13,18,20-23]. A centrifuge (detox unit) with anti-twister mechanism is used in the blood cell separation technique which causes very little damage to the cells. There are many features that render this fractionation technique unique. In this technique, no density gradient chemicals or chemical sedimentation agents are used. The experiments have been performed in a seal-less centrifuge using a trapezoidal chamber head.

The experiments were performed in order to find the optimal regime for a good blood cell separation in the first stage. This was done because we expect a large variation in blood characteristics such as hematocrit (HCT), red blood cell (RBC) concentration, white blood cell (WBC) concentration, hemoglobin etc. from one patient to another, which will cause various separations to take place.

The critical parameters for a good separation are HCT, centrifuge speed, withdrawal rate and residence time of blood in the chamber. A method has to be developed that can account for separation efficiently on a patient to patient basis. This device can be directly hooked to the patient and blood processing can be done on-line.

Our technique of separation has proven to be very efficient and the separation that previously was obtained via a six stage process [22,23] can now be achieved in a single stage.

The separation of the first stage provides us with WBC enriched plasma which can be further used for multistage separation. The chamber used in the first stage is also utilized in the second stage separation. Multistage separation is expected to give a separation of differential WBCs which in turn could be studied for healthy and unhealthy white cells.

In the first stage, the initial conditions were varied for the assay and the results were compared. The main parameter which was altered for different observations was the hematocrit (HCT) of the blood. In latter experiments second stage separation was also performed, that is,

blood samples enriched with the white blood cells were collected from separation of the first stage, and spun again in the centrifuge to gain further purity of the white cell collection.

The results of first stage separation and second stage separation presented different graphical outputs.

#### Sedimentation Theory in application to blood processing separation technique.

This section follows the mathematical development of Van Wie [18] and Sofer and Van Wie [21-25]. The Stokes' equation is the fundamental principle of sedimentation theory [18]. It describes sedimentation in dilute suspensions. It is very useful in predicting the particle velocity or sedimentation rates. Under a centrifugal force field, the value of the sedimentation rate is given by:

$$S_i = \frac{2r^2(\rho_i - \rho_f)\omega^2 r_c}{9\mu}$$

where :

$S_i$  = terminal sedimentation velocity of species i;

$r$  = the radius of species i ;

$\omega^2 r$  = angular acceleration exerted due to the centrifugal force ;

$\rho_i$  = density of species i ;

$\rho_f$  = plasma density ; and

$\mu$  = viscosity of the suspending fluid medium .

Modifications are made in this basic formula to include blood characteristics. The Hawksley-Vand modification of the Stokes' equation is used to explain individual blood cellular species.

When whole blood is allowed to stand in a test tube, gravity causes settling of various blood fractions into respective layers. The red blood cells have a higher density compared to other suspended particles in blood, and they also tend to adhere to each other forming rouleaux, which augment their settling to the bottom of the test tube.



The white blood cells, which are less dense form a buffy coat over the red cells. Plasma with some salt minerals, carbohydrates, lipids and proteins appears on the top.

The rate of sedimentation is speeded by imposing angular acceleration in the centrifugal process. The centrifugal speed is limited in order to prevent shear damage to the cells under the influence of very high rotating velocity.

## THEORY

First step is to study the sedimentation process of centrifuged, whole blood particles in general. Next, we will study the different cases by varying the HCT and holding the centrifuge speed and the withdrawal rates constant.

According to the fundamental principle of circular motion, centrifugal force increases as the radius increases. This is the reason the higher density particles get pushed towards the outer diameter of the chamber.

Applying this principle to separation of blood components, it is obvious that RBCs get pushed towards the outer diameter and displaced plasma along with some minerals move closer to the axis of the machine. After a certain duration of time, a distinct separation line is observed in the chamber between the packed red cells and yellow colored plasma. The WBCs form a buffy thin coat between the RBCs and the plasma medium.

The separation front is the interface between the plasma phase and cell phase. Static mode defines the duration of the batch process. The distance of this front from the inner side of the chamber increases in the static mode. As time progresses in the static mode, the separation front gradually moves towards the outer diameter of the chamber, until it reaches a specific position, after which it does not move. This phenomenon can be explained in the following manner.

Initially, RBCs move individually towards the outer side of the chamber and they collide with each other and clash against the chamber wall. The sedimentation velocity increases as rouleaux formation takes place along with the settling mechanism. When the RBCs have almost settled in their respective positions, they get packed together and cannot move any further. From that time onwards the separation front does not seem to move any further beyond that particular line of packed cells.

## Centrifuge Equipment

The centrifuge used for the purpose of blood cell separation was first developed by Ito and Kolbow at NIH. A new machine was constructed by Sofer's group at the University of

Oklahoma and brought to NJIT in 1986. The trapezoidal chamber head was designed Sofer and Camp of this laboratory. This design is based on Boycott's principle of tapering (slanting) edges. The centrifuge speed can be varied between 500 to 2000 rpm. For our experiments, we maintain the speed constant at 900 - 1000 rpm. The essential attributes of the blood fractionator are a dynamic bearing balance system to reduce vibrations, Tygon tubing for the entry and exit of blood to and from the chamber, a peristaltic pump for control of the blood process stream, and an anti-twister mechanism to connect the tube directly to the rotating centrifuge rotor in order to prevent any twisting or shearing of the tube and damage to the cells.

### Experimental Procedure

Bovine blood has been used for the experimental analysis because of two reasons - cow blood is less hazardous to work with than human blood, and it is easily available.

Blood was stored at a low, constant temperature in the refrigerator. Before feeding it in the chamber it was stirred properly. Initially the blood was strained through cheese cloth which was slightly soaked in anti coagulant diluent (ACD) solution to remove external impurities. The blood chamber in the centrifuge and the connecting tubes were rinsed with ACD solution two to three times before the run. A constant volume of blood (21 ml) was fed through the tube which enters the chamber at its midpoint. The initial parameters, which remained constant throughout the run were defined for the experiment. Centrifuge operating speed was maintained close to 1000 rpm for the runs. The speed of the machine has a bearing on the time required for the separation. Increasing the speed exerts more centrifugal force which reduces the time to achieve separation.

The flow of blood into and out of the chamber can be regulated; in our runs it has been maintained constant at 10.4 ml/min at a motor setting of 150 rpm.

Blood was spun in the chamber till the separation front (distinct red cell layer and yellow plasma layer) became prominent to the eye and was from 0.5 cms to 2.5 cms in width. This width depended on the static spin duration of blood in the chamber.

Following this static mode with no input to the chamber or output from the chamber, was a dynamic mode. In the dynamic mode the chamber inventory was withdrawn. Blood from the bottom of the chamber was extracted and collected in equal amounts (approximately 3 ml) in 7 test tubes. Because the blood withdrawal port was on the outer side of the chamber the first few samples of blood had a high concentration of RBCs. As the withdrawal progressed less viscous and lower HCT blood was collected and at the very end plasma was left in the last test tube.

Volume and time required for each sample were noted. The samples were kept on ice. Each sample was stirred using a motorized propeller. The HCT of the samples was determined

using the microhematocrit technique. RBC, WBC and platelet counts in each sample were determined with a Coulter Counter model ZBI.

Results obtained from the experiments were graphed. Fraction of individual cell type withdrawn was plotted against the fraction of total blood volume withdrawn.

### EXPERIMENTAL RESULTS

Good separation is very critical and it depends on a number of factors such as HCT, batch duration, distance of the separation front from the inner diameter of the chamber, centrifuge speed and the pump withdrawal rate.

In our study of assays, the speed and the withdrawal rate have been kept constant. The duration of the run is a variable factor dependent on the separation front. This in turn is dependent on the HCT of blood. If the HCT of the blood is low, then the separation front is visible much faster and the plasma phase is greater volume than the cell phase. On the other hand, if the HCT is very high, the spin duration is longer and the plasma phase is smaller compared to the cell phase.

The following table shows the initial parameters of each run and the results of their separation.

HCT of blood	SPEED (rpm) of the centrifuge	TIME (sec) duration of the static portion of the run	PARTITION (%) volume	COMMENTS Blood returned to the patient can have RBC% WBC% of the total blood volume.
49	900	4500	65	85 35
44	900	5600	78	92 40
42.75	900	4560	85	98 18
40.4	900	4500	80	96 28
40.7	1100	1980	40	78 8
37	910	4740	50	88 36
31.8	900	4500	40	88 30
30.3	950	3300	35	76 35
27.9	900	3000	25	56 20
19.9	900	3300	25	90 38
9.9	900	1800	18	84 28
8.3	900	1800	15	84 30
5.4	900	1800	10	80 58
4.2	900	2100	10	84 76
2.0	900	1800	10	88 78
2.11	1000	3060	60	96 62

The effect of HCT on cell separation is visualized by plotting percentage fraction of red blood cells and white blood cells v/s the volume of blood recovered from the chamber.

After performing a number of experiments at different HCTs, it was found that the optimal operating condition for the centrifuge occurred at HCTs in the range of 40%-45%. The machine operating speed was 900 - 1000 rpm, withdrawal flow rate was 10.4 ml/min at a motor setting of 150 rpm.

The maximum difference between the ordinates of RBC and WBC concentrations on the graph defines the partition for the blood samples. To the left of this partition, blood samples are rich in RBCs and to the right of this partition the blood samples are rich in WBCs.

Comparing two graphs in the optimum regime, we can see from Figure 5.1 that the partition at the sixth cut is excellent. To the left of the sixth cut, there is RBC enriched blood which can be given back to the patient. That is, 85% of the blood volume used for the experiment can be returned to the patient. The remaining blood is highly concentrated with WBC which can be diluted with plasma and recycled for further separation. The static mode spin duration for this run was 4500 sec.

In Figure 5.2, the experiment has the same initial conditions as the previous run except for the batch duration. The static mode was much shorter. The graph shows an excellent partition at the third cut, which implies that only 40% of the blood volume can be given back to the patient. The blood returned to the patient has 80% of the total RBCs and 20% of the WBCs.

From the two graphs we can conclude that the residence time of blood in the chamber is an important factor for determining the percentage of blood to be returned to the patient. In other words, the partition for the blood volume (difference in the ordinates) is dependent on the batch duration.

Comparing the plots of different HCTs (shown in Figures 5.3 - 5.13), some prominent observations are:

1. As HCT is decreased from 45% to 4%, the area within the curve becomes smaller.
2. As HCT is decreased, the required static residence time of blood in the chamber also decreases.
3. For high HCTs, the WBC slope rises very gradually in the beginning and then suddenly shoots up; whereas, as HCT is lowered, the slope becomes steeper and WBC curve runs nearly parallel to the RBC curve.

A closer evaluation of these graphs shows a partition for the separation of the RBC and WBC blood samples. In most of these graphs the partition is seen in the second or the third cut.

When the HCT is lowered to 4.0%, there is an inversion of the WBC and RBC curves. A point of intersection between the two curves is seen at this HCT ( Figure 5.14).

At very low HCTs the RBC concentration is low in the suspended plasma medium. There is no rouleaux formation and since WBC are bigger in size than RBC, they tend to settle faster.

In Figures 5.15 and 5.16, HCT at 2%, it is seen that the WBC curve is above the RBC curve. It is clearly seen in both graphs that an inversion takes place - WBC, not RBC, are the heavy components. In the first graph, the spin duration was only 1800 sec, whereas in the second graph the spin duration was increased to 3600 sec which resulted in a better separation. Once again it is proved that time plays a critical role in the overall separation of the blood components.

The other difference between the two graphs, which is very important to note, is the formation of the blood sample in achieving the desired HCT prior to the run. For Fig. 5.15, bovine blood was directly diluted with plasma to get HCT of 4%. For Fig. 5.16, the blood samples rich in WBC were collected from the first stage and then diluted to get the desired HCT. The WBC/RBC ratio was higher in the second graph (Fig. 5.16).

This means recycling of the desired blood fraction from the first stage gave a better separation. This was first proposed earlier [24-25] and is herein confirmed.

The second stage gives more purity of WBC concentration and it is one step towards multi-stage centrifuging technique for separation of differential white blood cells.

In conclusion we can say that a search for the optimum condition of the centrifuge is very important in order to achieve efficiency in separation.

For the first stage, the favorable operating regime was found to be in the HCT ranging from 40% - 45%. Centrifuge speed for separation should be limited to prevent shear damage to the cells. The change in withdrawal rate does not have a very great bearing in the overall separation. Nevertheless, it must be kept constant and within the range to allow continuous flow of blood to and from the chamber. If the flow rate is increased too high then the blood due to its high viscosity, gets plugged in the tubing while flowing out of the chamber.

Residence time of blood in the chamber is the key operating factor for the partition between the blood samples. The occurrence of this partition in the graph determines the amount

of blood that is rich in RBC and can be given back to the patient, and the amount of blood rich in WBC which can be further processed. The WBC enriched blood collected from the first stage provides the input for the second stage. The second stage gives further purity of WBC concentration. The multistage separation would result in the separation of differential components of the white blood cells.

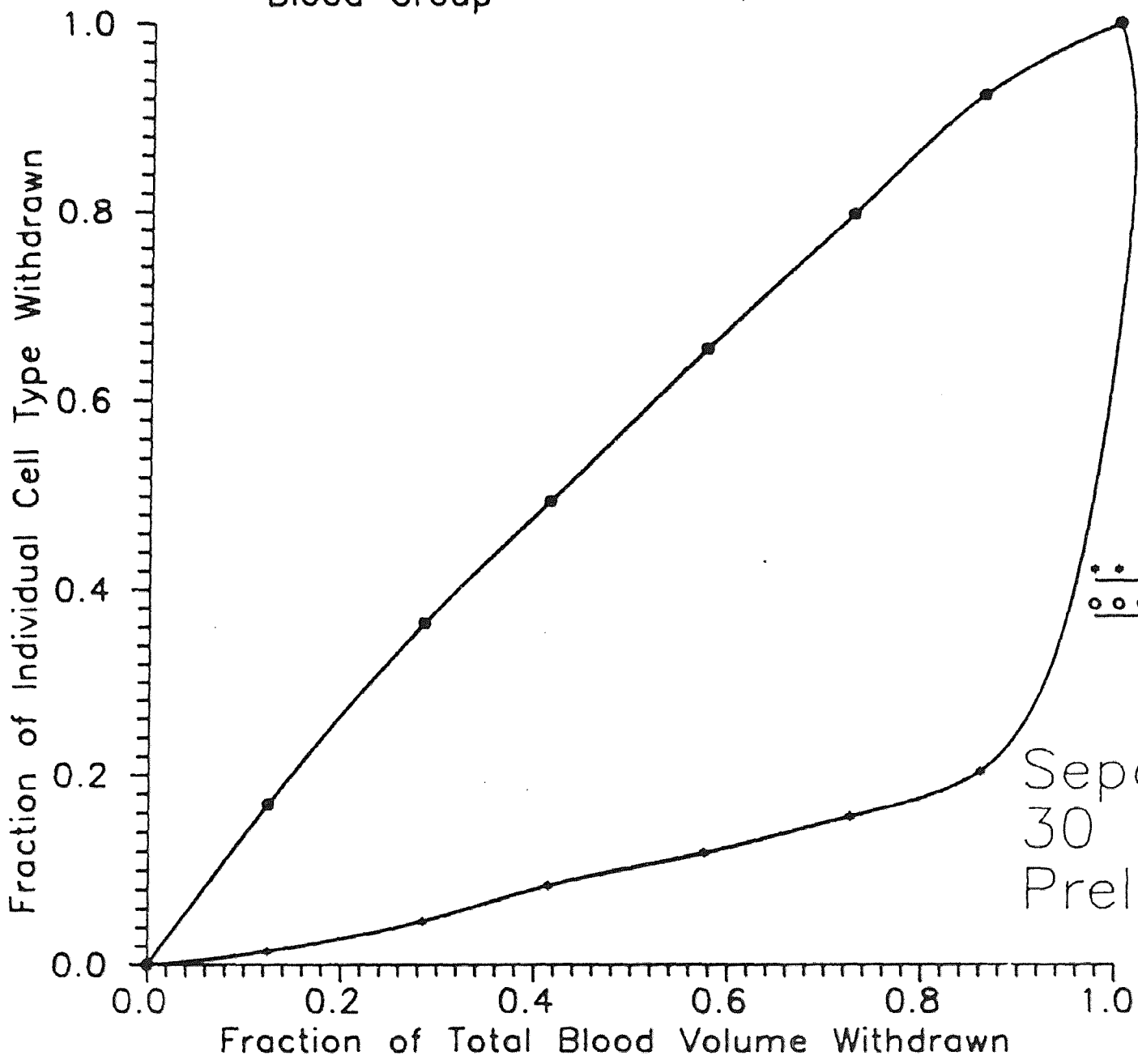
This study provides a basis for the separation technique and further research in this field is expected to give better results in the blood cell separation.

Recently experiments with human blood (donated by Sam Sofer) were performed at UMDNJ. The separation of white blood cells in human blood was achieved faster as we had predicted, compared to cow's blood. Also the separation on the whole was better.

We intend to exploit this technique further to process human blood and achieve separation of differential white blood cells. With the basic knowledge of fractionation, modifications can be implemented and success can be achieved in the separation of infected white cells from the healthy white cells which is proposed by us for the cure or at least longer survival of an AIDS patient.

NJIT Biotechnology Laboratory  
Blood Group

Vampire III.  
Stage I.

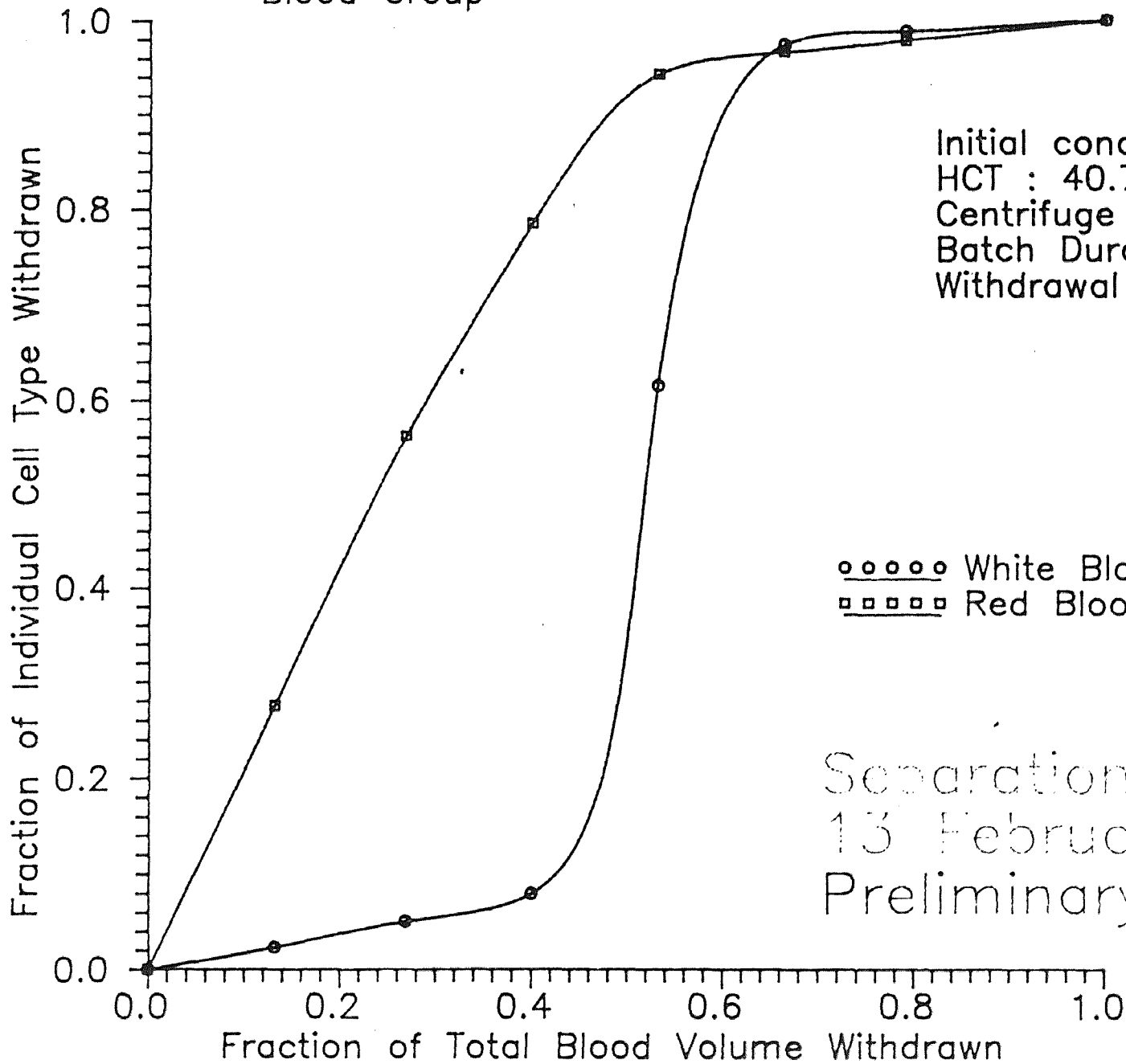


Initial conditions  
HCT : 42.75  
Centrifuge Speed: 90  
Batch Duration: 456  
Withdrawal Rate: 200

..... White Blood Cells  
..... Red Blood Cells

Separation B8909  
30 Sept 1989  
Preliminary Exper

NJIT Biotechnology Laboratory  
Blood Group

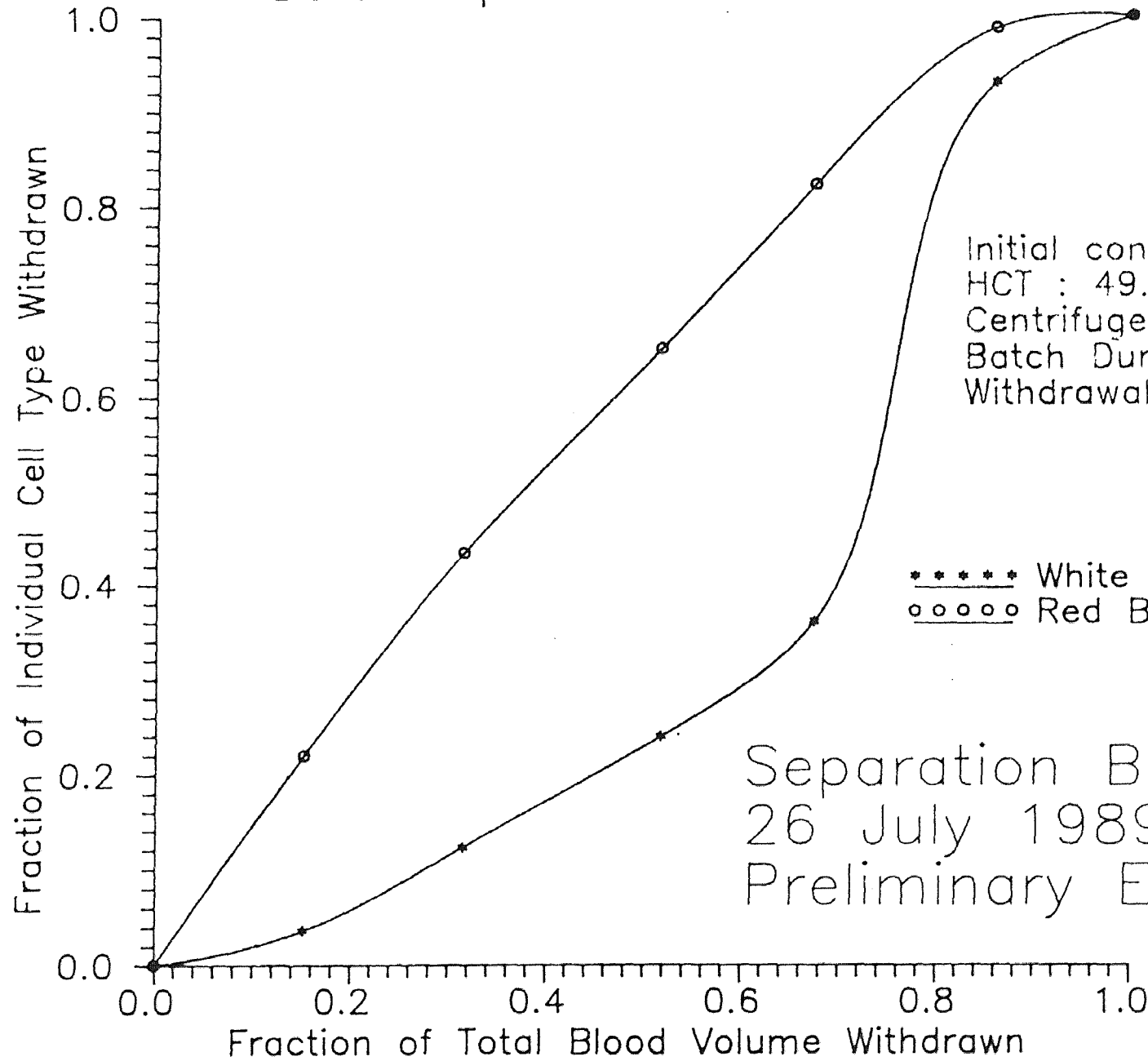


Initial conditions  
HCT : 40.7  
Centrifuge Speed: 1100  
Batch Duration: 1980  
Withdrawal Rate: 200

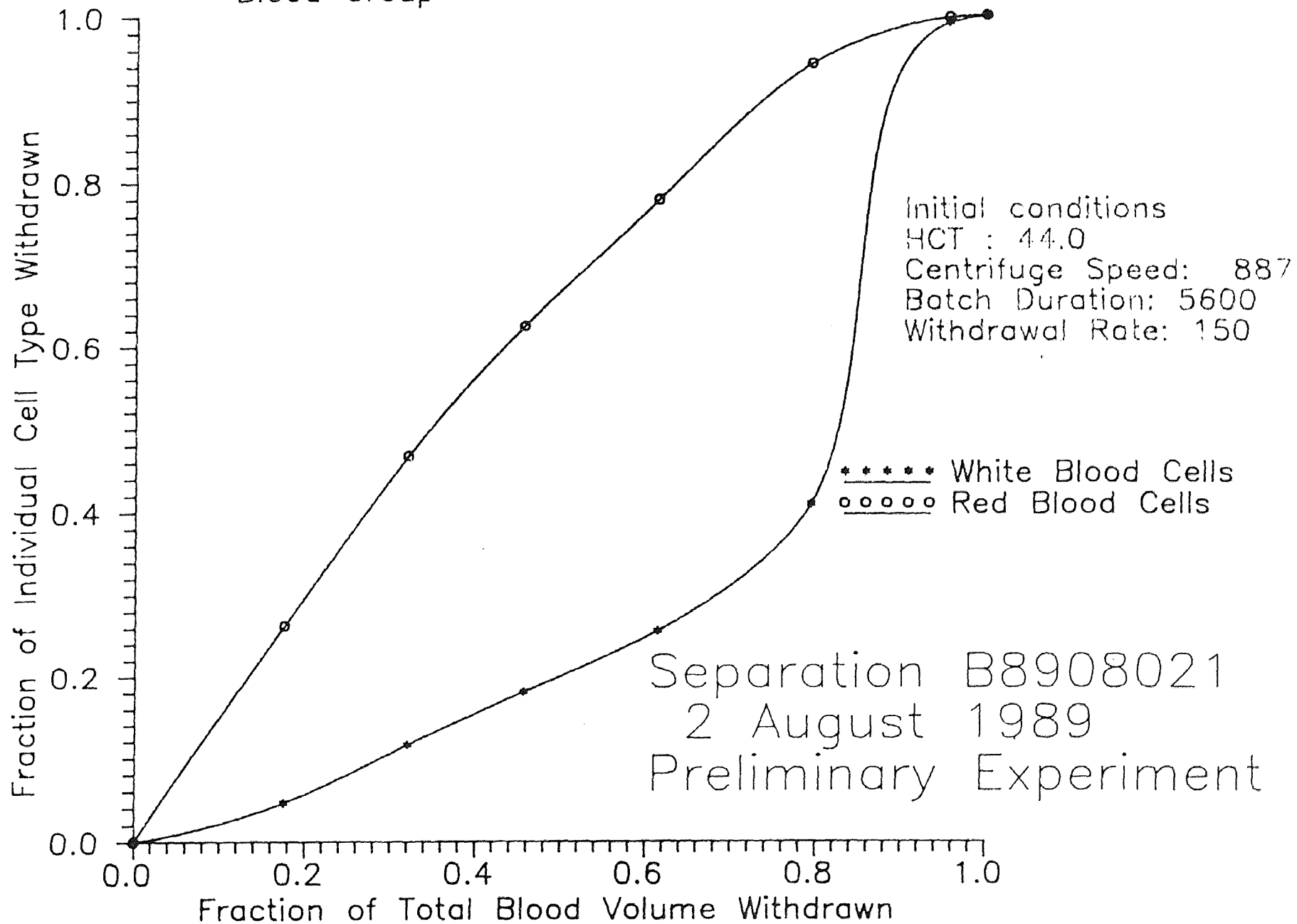
○ ○ ○ ○ ○ White Blood Cells  
□ □ □ □ □ Red Blood Cells

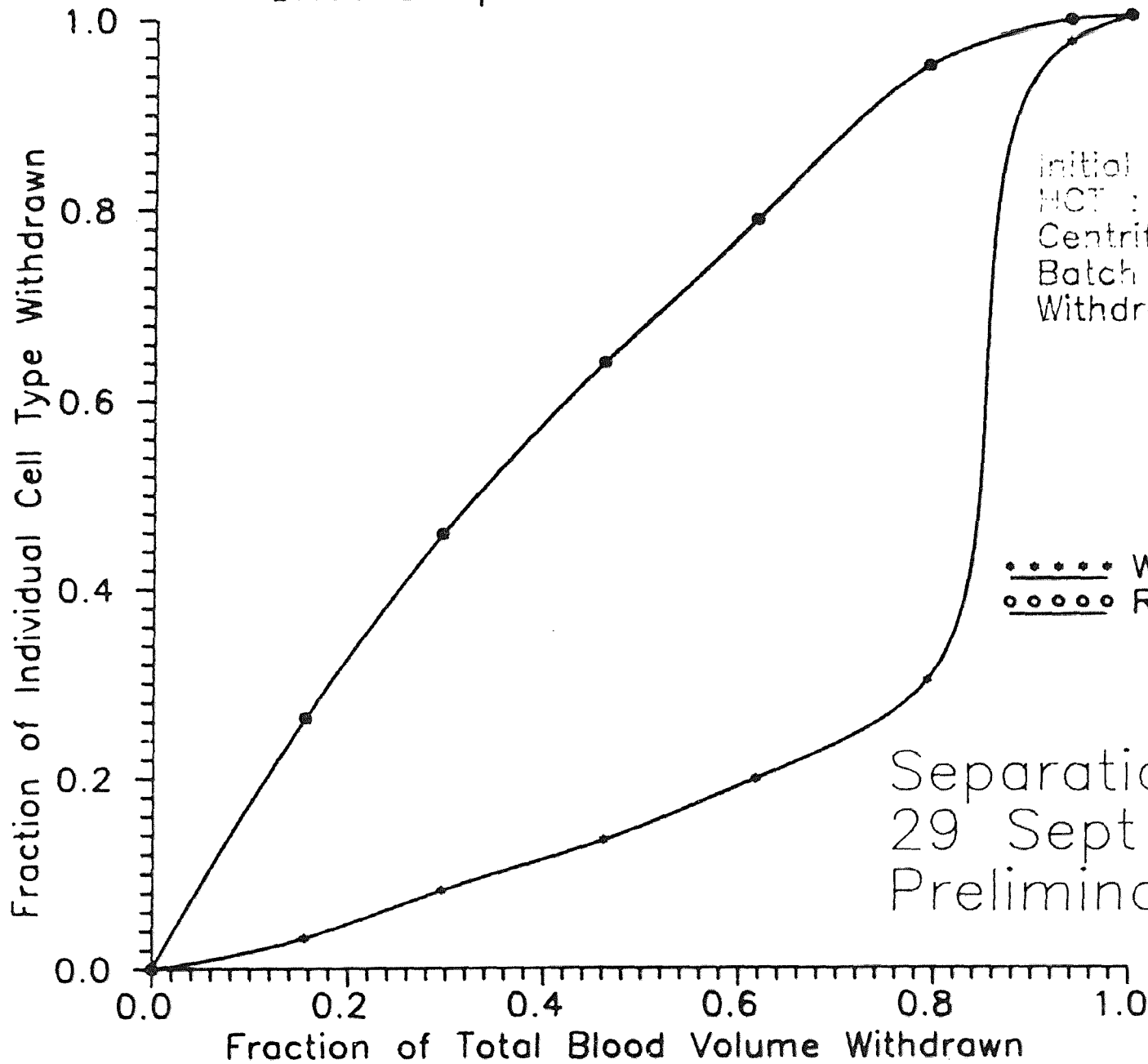
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13 February 1989  
Preliminary Experiment



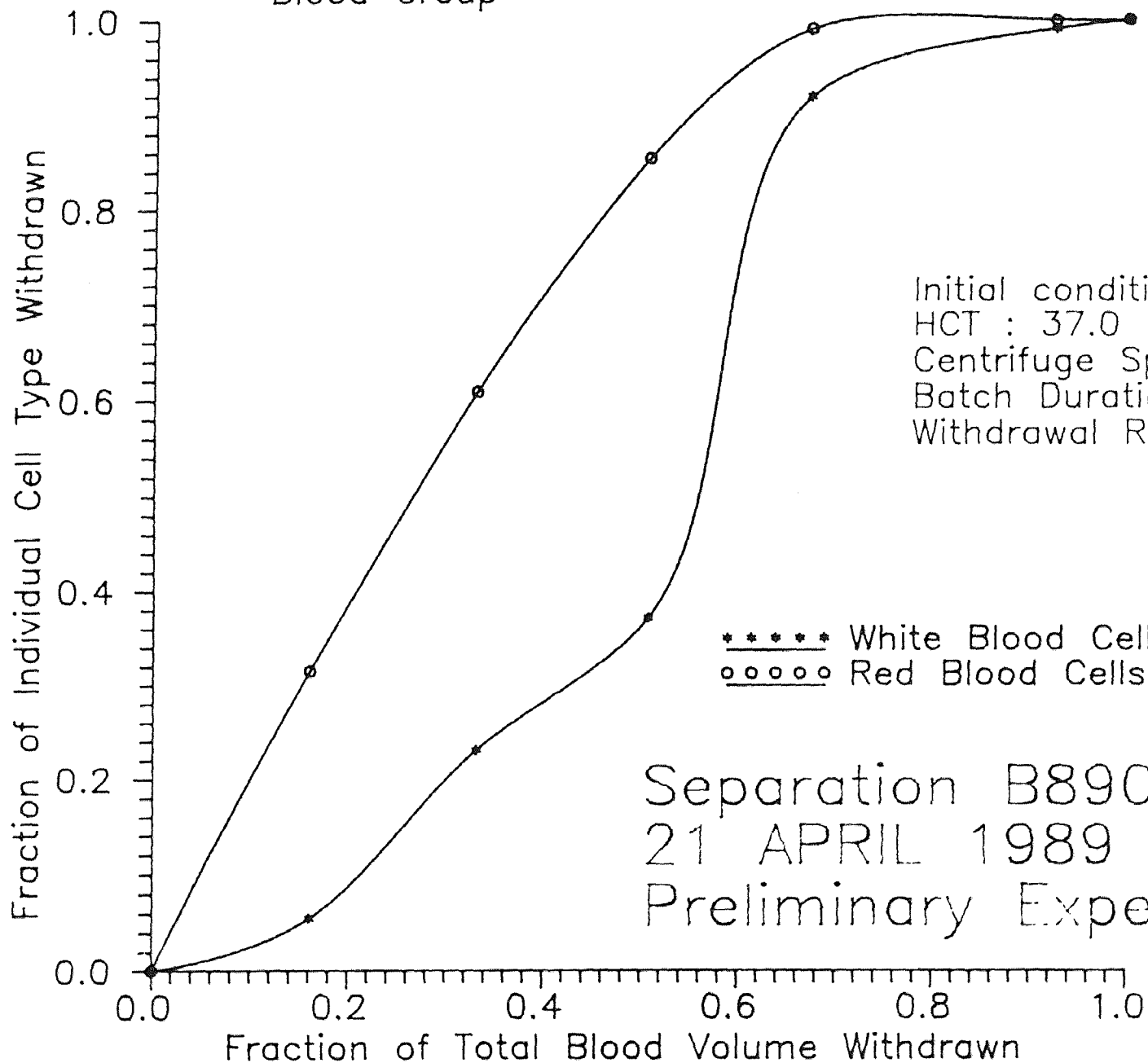


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26 July 1989  
Preliminary Experiment





Separation B8909291  
29 Sept 1989  
Preliminary Experimer

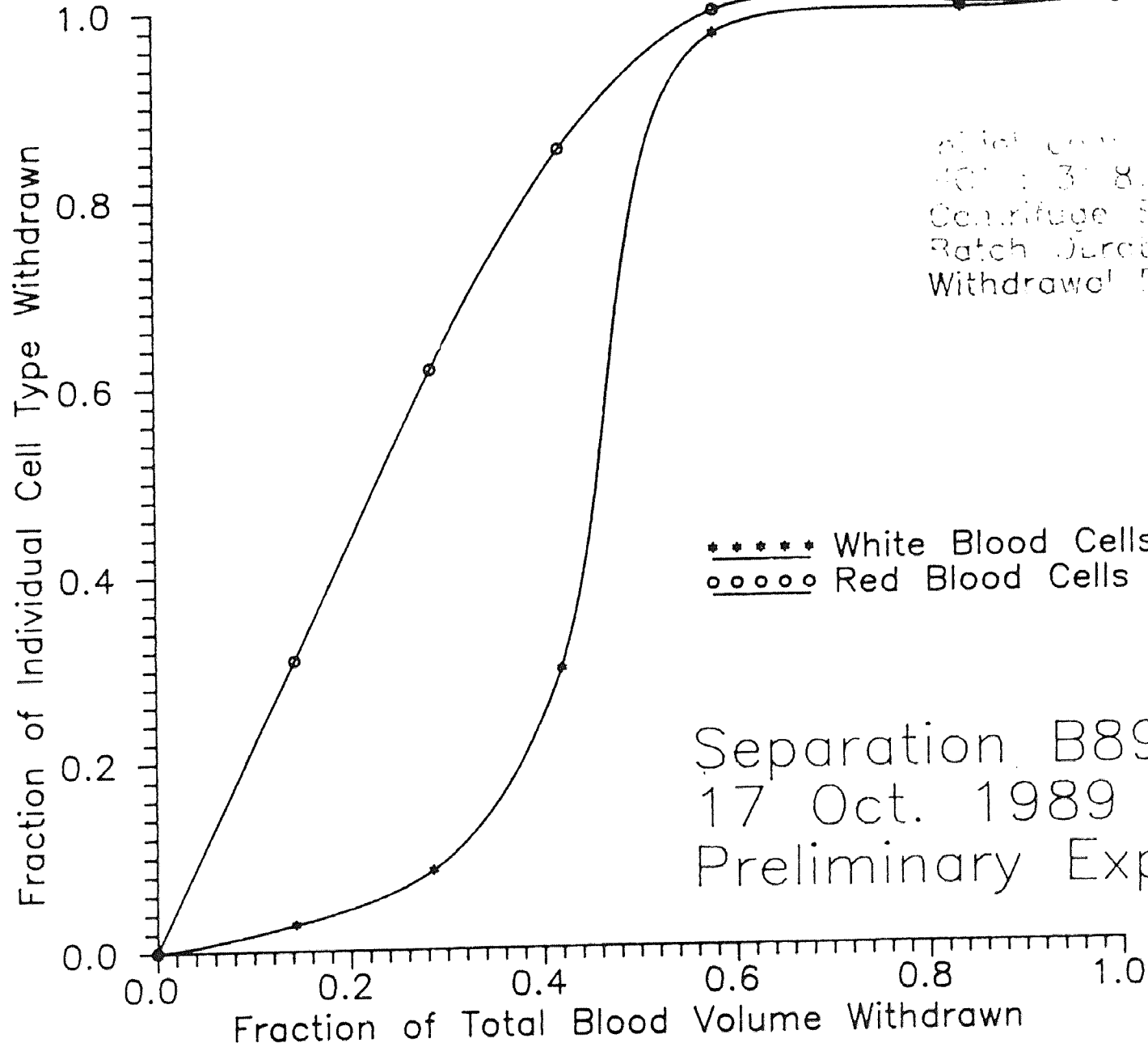


Separation B8904211  
21 APRIL 1989  
Preliminary Experiment

NJIT Biotechnology Laboratory  
Blood Group

Vampire III.

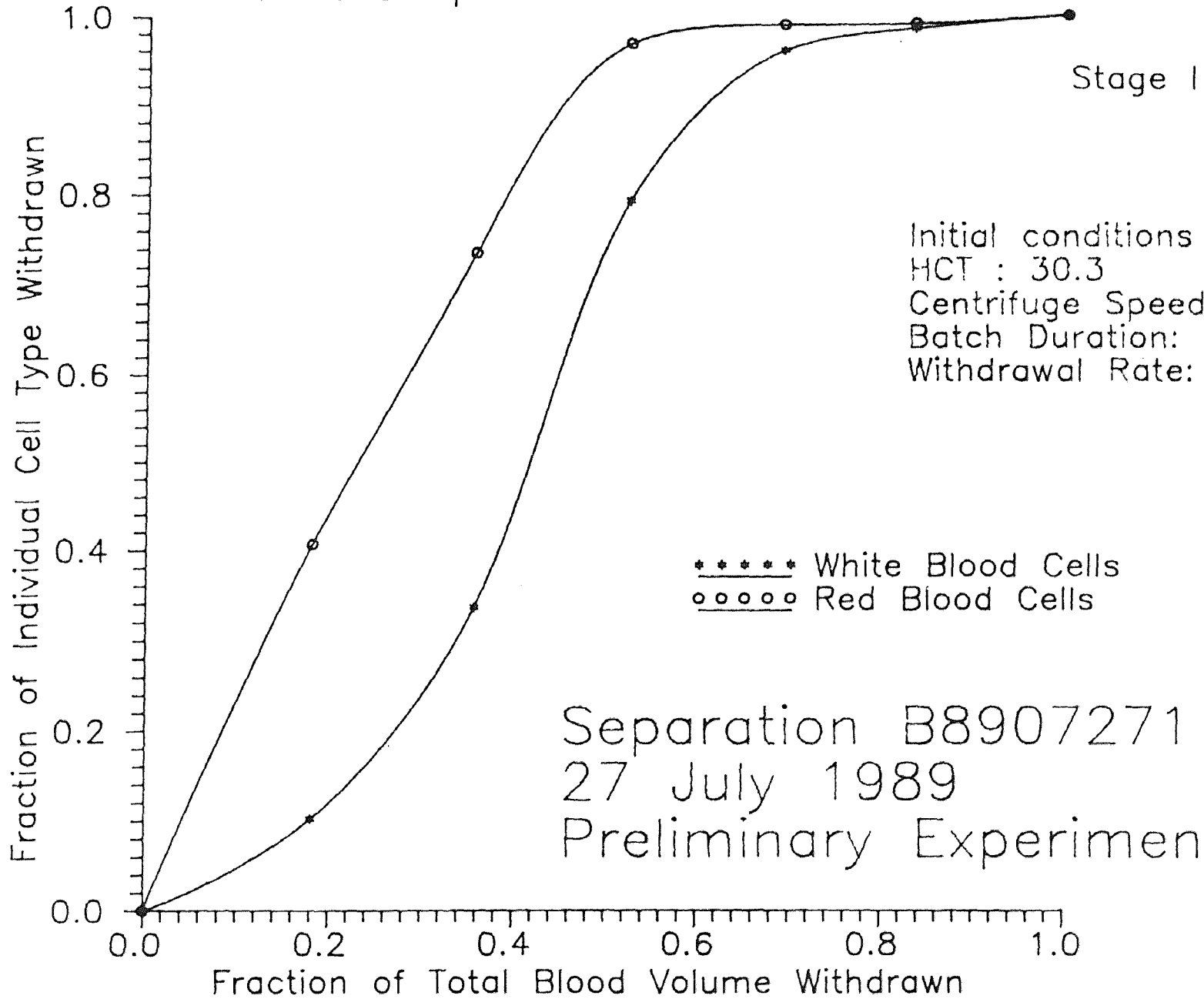
Stage I.



Initial Cell Count:  
RBC: 3.8  
Centrifuge Speed: 900  
Batch Duration: 4500  
Withdrawal Rate: 200

Separation B8910171  
17 Oct. 1989  
Preliminary Experiment

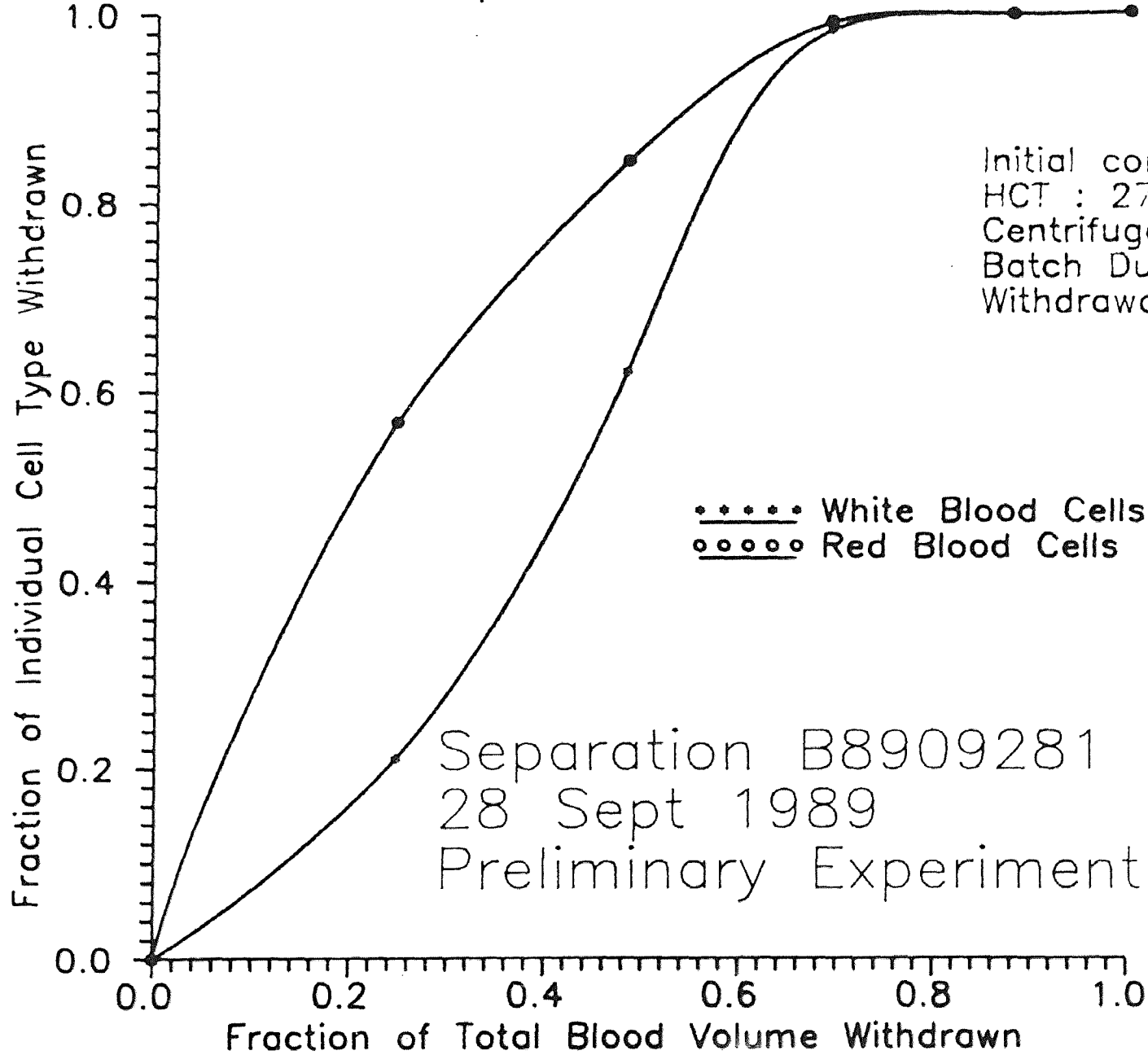
NJIT Biotechnology Laboratory  
Blood Group



NJIT Biotechnology Laboratory  
Blood Group

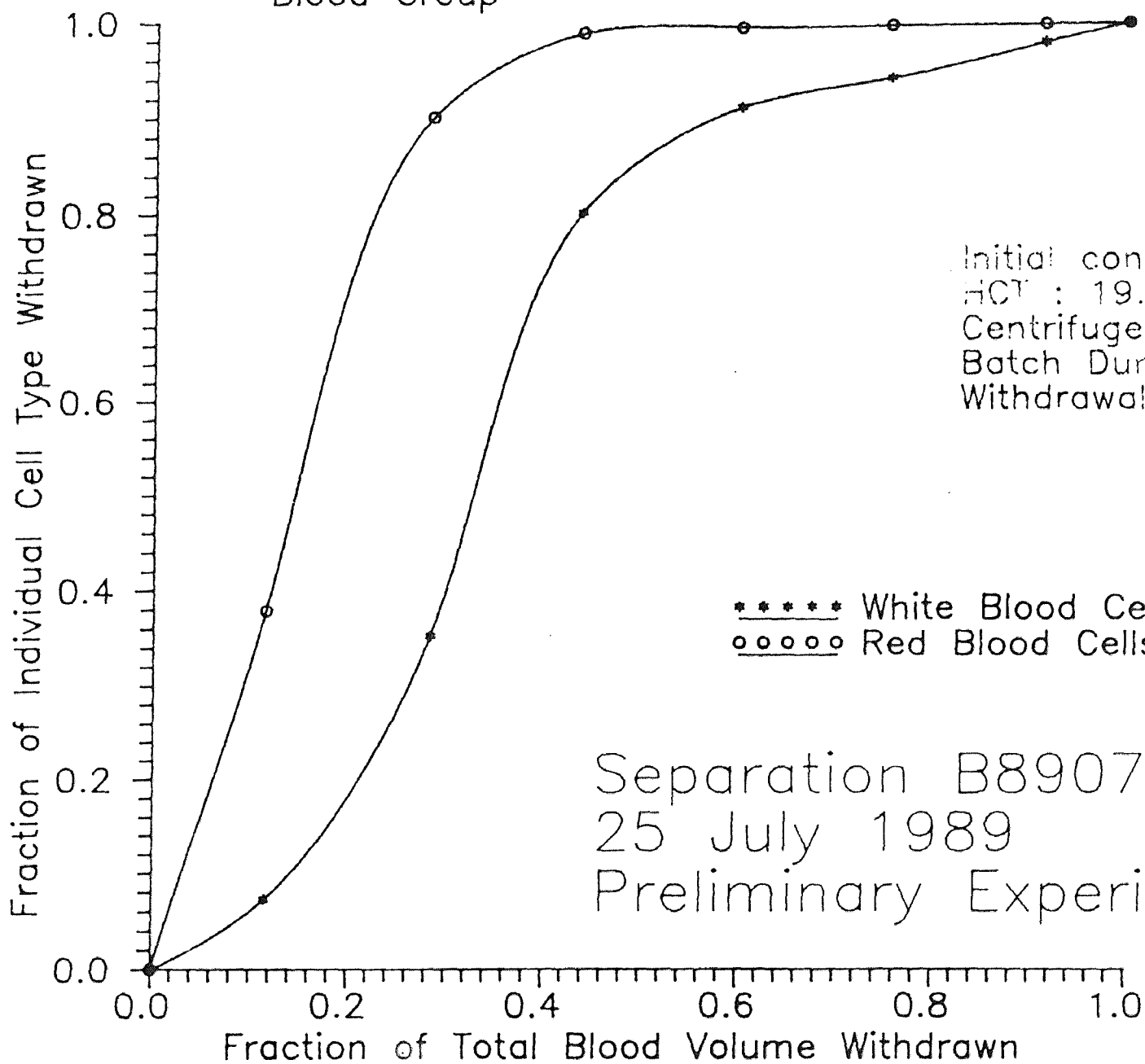
Vampire III.  
Stage I.

Initial conditions  
HCT : 27.9  
Centrifuge Speed: 900  
Batch Duration: 3000  
Withdrawal Rate: 200



NJIT Biotechnology Laboratory  
Blood Group

Stage 1



Initial conditions  
HCT : 19.9  
Centrifuge Speed: 910  
Batch Duration: 3300  
Withdrawal Rate: 150

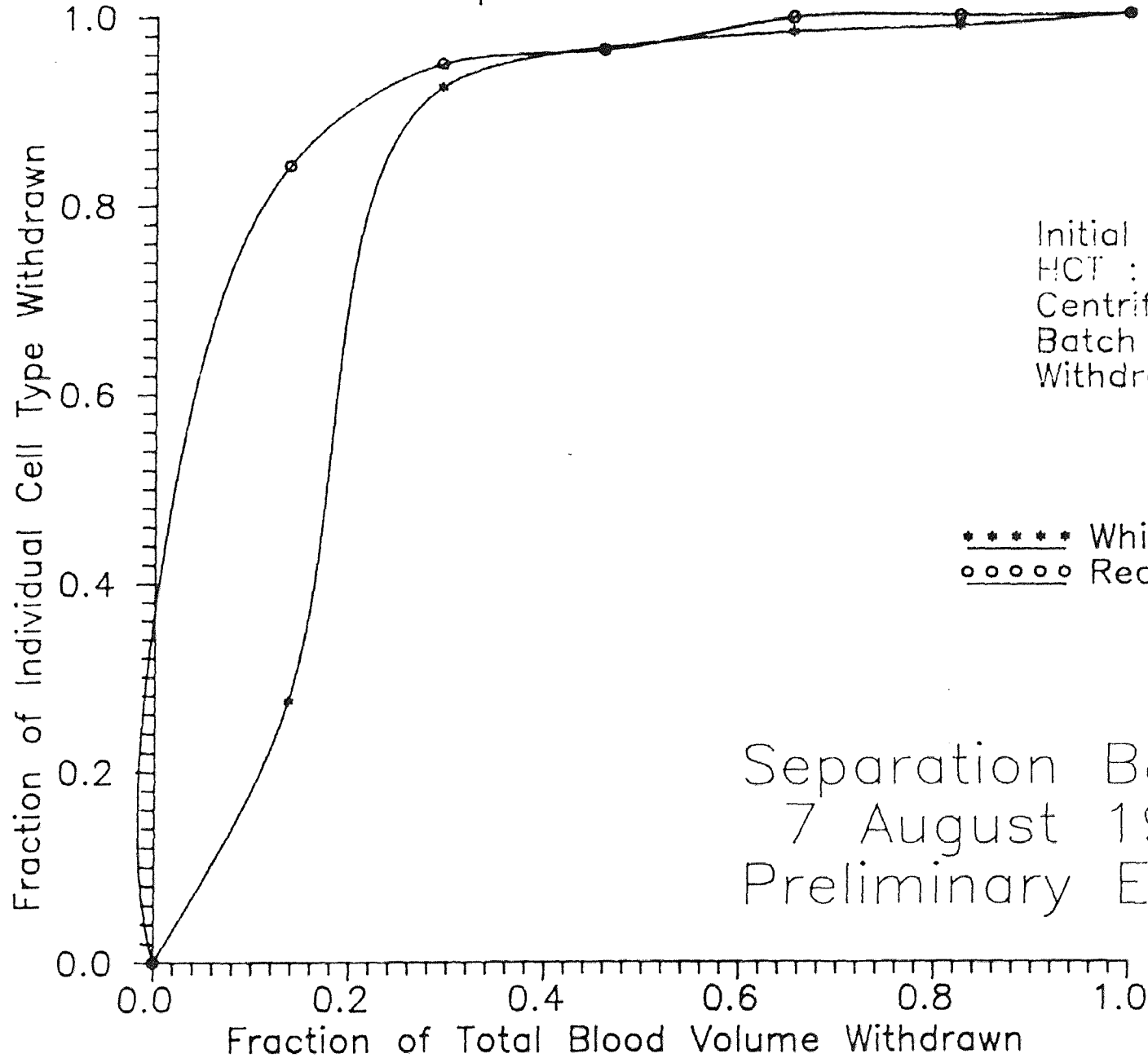
\*\*\*\*\* White Blood Cells  
ooooo Red Blood Cells

Separation B8907251  
25 July 1989  
Preliminary Experiment



NJIT Biotechnology Laboratory  
Blood Group

stage 1



Initial conditions

HCT : 9.9

Centrifuge Speed: 890

Batch Duration: 1800

Withdrawal Rate: 150

\*\*\*\*\* White Blood Cells

ooooo Red Blood Cells

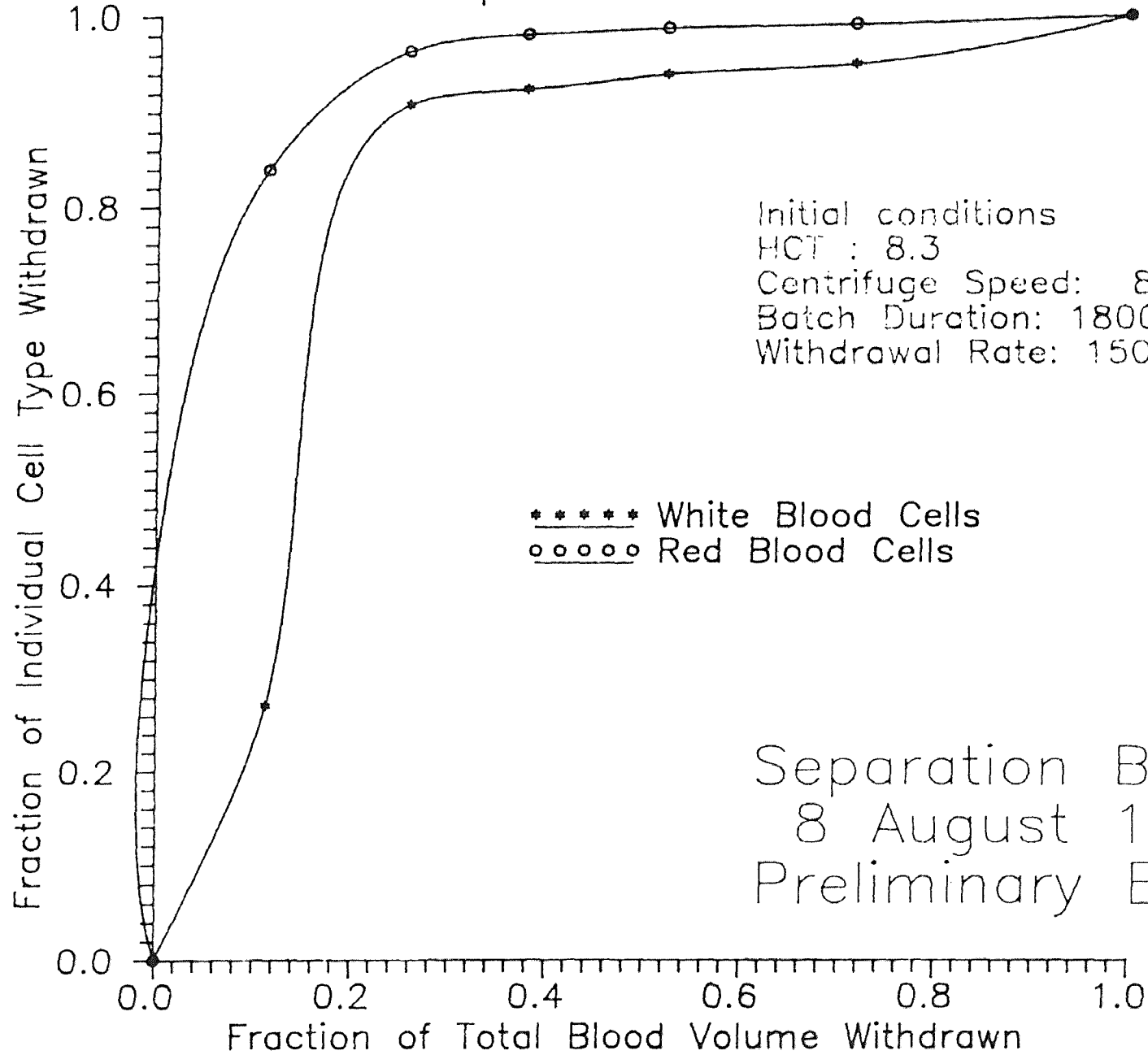
Separation B8908071

7 August 1989

Preliminary Experiment

NJIT Biotechnology Laboratory  
Blood Group

stage 1



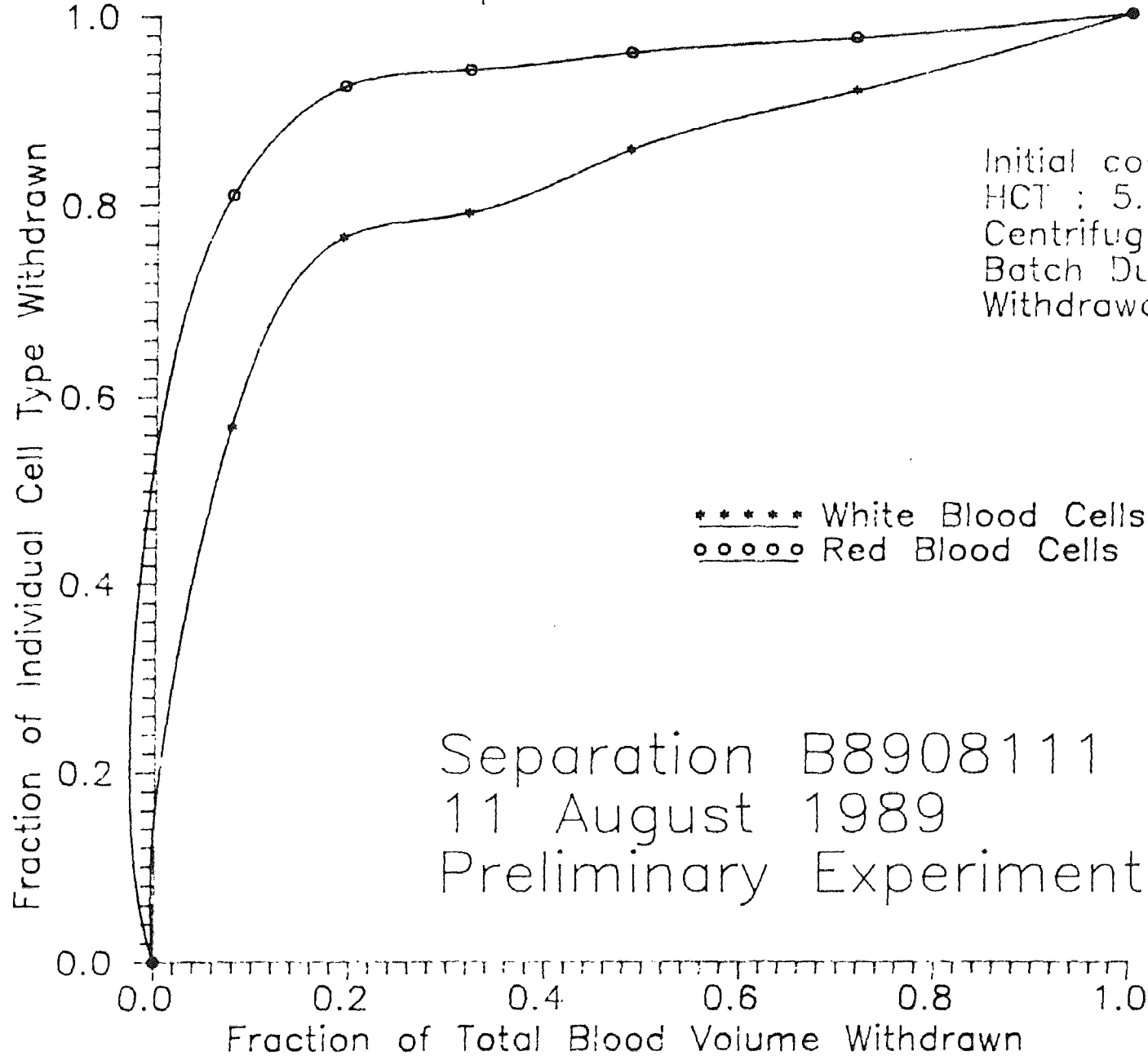
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8 August 1989  
Preliminary Experiment

NJIT Biotechnology Laboratory  
Blood Group

Vampire III.

Stage I.

Dilute blood



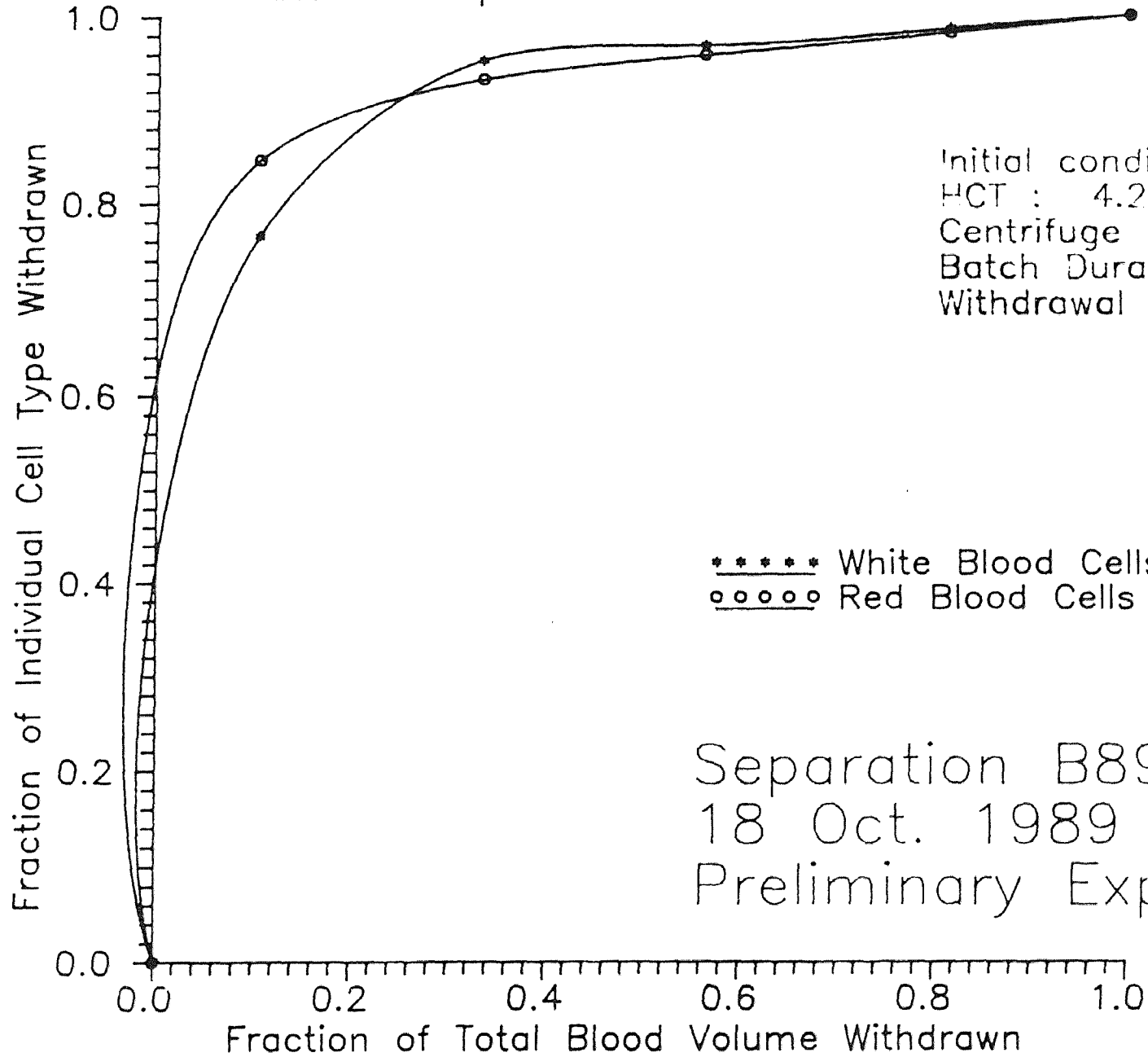
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Preliminary Experiment

NJIT Biotechnology Laboratory  
Blood Group

Vampire III.

Stage II.

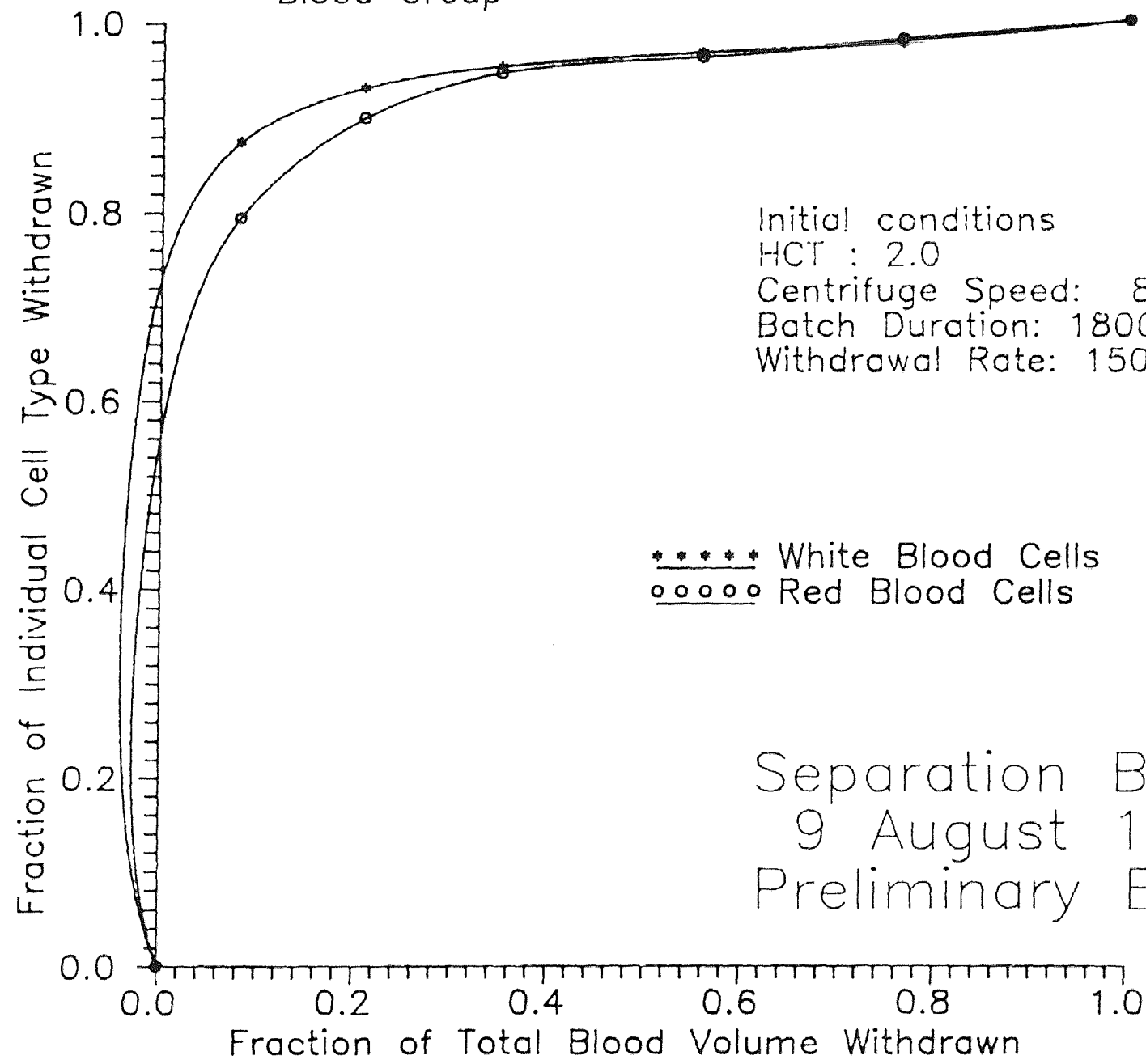
Initial conditions  
HCT : 4.20  
Centrifuge Speed: 900  
Batch Duration: 2100  
Withdrawal Rate: 200



Separation B8910181  
18 Oct. 1989  
Preliminary Experiment

NJIT Biotechnology Laboratory  
Blood Group

stage 1  
Dilute Blood



Initial conditions  
HCT : 2.0  
Centrifuge Speed: 870  
Batch Duration: 1800  
Withdrawal Rate: 150

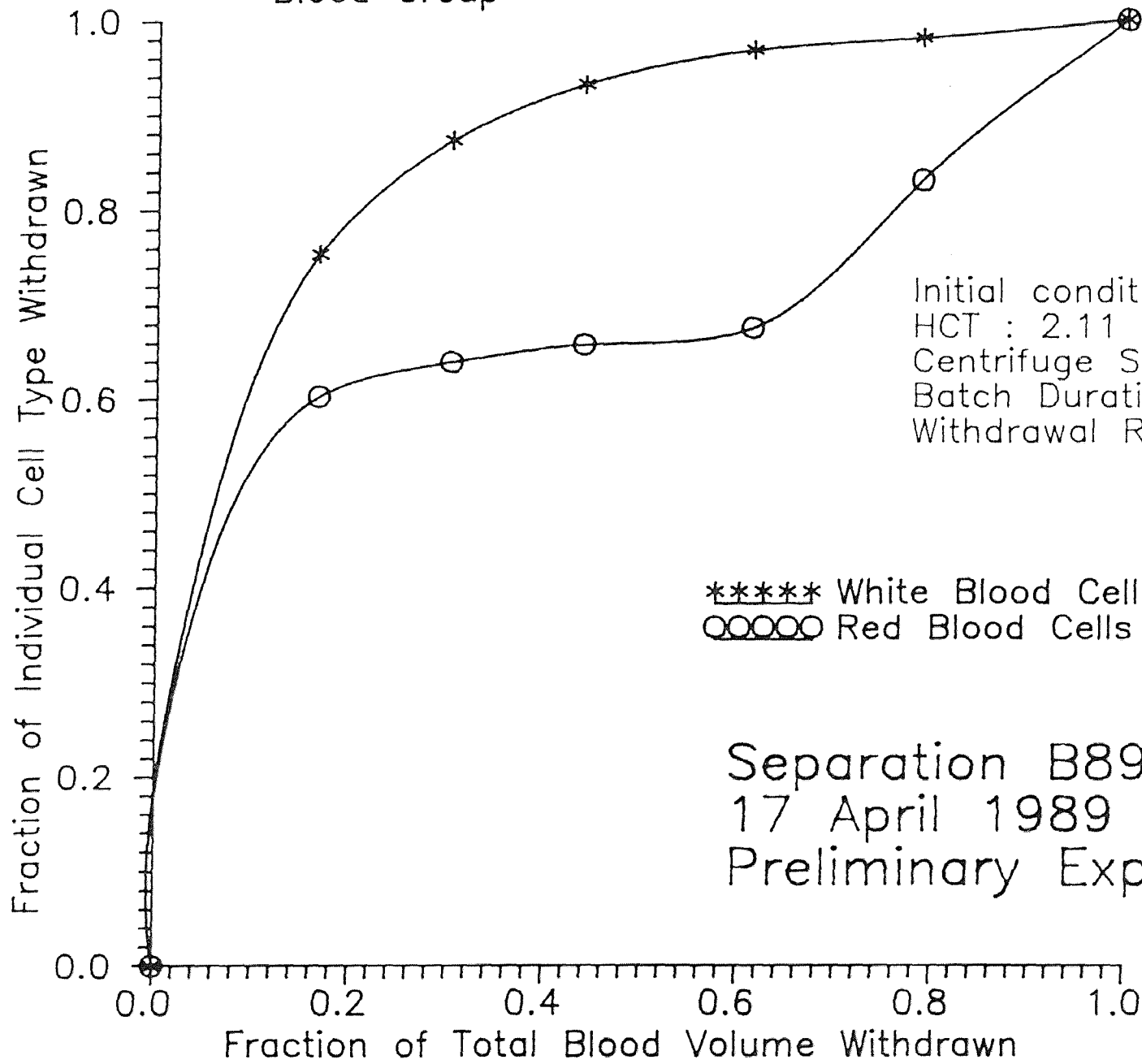
\*\*\*\*\* White Blood Cells  
ooooo Red Blood Cells

Separation B8908091  
9 August 1989  
Preliminary Experiment

NJIT Biotechnology Laboratory  
Blood Group

vampire III.

Stage II.



Initial conditions  
HCT : 2.11  
Centrifuge Speed: 1000  
Batch Duration: 3060  
Withdrawal Rate: 75

\*\*\*\*\* White Blood Cells  
OOOOO Red Blood Cells

Separation B8904171  
17 April 1989  
Preliminary Experiment

# CHAPTER 6

## SIMULATION RESULTS AND DISCUSSION

Modeling and simulation are being used in various ways to explore the intricacies of the immune system. Our starting point is that one needs to have a good understanding of the elementary building blocks before beginning work with larger simulation models.

The present state of biological knowledge does not yet give us a reliable means of predicting the manifestation of AIDS after HIV transmission. However, a simulation approach based on a mathematical model can be quite useful. This thesis describes a basic model of AIDS dynamics in the form of differential equations with the application of a FORTRAN program. The model consists of several parameters which describe the immune changes accompanying the progression of AIDS.

The solution of the differential equations was achieved by applying the Gear algorithm [16,17]. The simulation was done on a personal computer (PC-80286). The results presented in this paper are discussed within the framework of the Walter Reed classification system [7]. The figures are plotted from the simulation calculations, where HIV is the only cause of death. Table 2 shows the different stages in Walter Reed classification based on T4 cell counts.

TABLE 2

WALTER REED CLASSIFICATION	
STAGES	T4 CELL COUNT
Stage 0	800-1000
Stage 1	> 400
Stage 2	> 400 (chronic adenopathy)
Stage 3	< 400
Stage 4	< 400
Stage 5	< 200 (anergy)
Stage 6	< 100 (full blown AIDS)

The Walter Reed classification begins with Stage 0. This stage is exposure to HIV. In the model exposure corresponds to a known initial concentration of the virus, 10 virus cells/ $\mu$ l. The main reason for initializing is that we have to force the mathematical model to respond to the presence of the virus.

Figure 6.1 and 6.2 show the rise in the concentration of the virus with respect to time. As seen in Figure 6.1 (microscopic view), the concentration of virus in the beginning is high due to the initial transmission. When the body is challenged with the virus, the immune system activates and resists the foreign particles. The concentration of the virus decreases. The individual is said to suffer from viremia. The disappearance of the virus indicates that initially the immune system functions effectively.

From six weeks to a year after the transmission of the virus, antigen concentration increases for a short while and then decreases, going back to zero. This small “blip” represents the acute infection in the victim which is the Stage 1 in the progression of AIDS. Following this “blip” is the latent phase of the antigen. This is Stage 2, in which the patient suffers from chronic lymphadenopathy. The antigen is hidden in the T4 helper cells and cannot be detected by conventional methods like ELISA or Western Blot. It lies dormant indefinitely in T cells, inextricable from the cell and hidden from the victim's immune system. The antigen stays quiescent for a long period and then starts to multiply within the cells when activated. The beginning of Stage 3 is defined by a persistent drop in the T4 cell count to less than  $400/\mu\text{l}$ . Stage 4 follows a few months later. The T4 cell count drops persistently lower. It can go below  $50 \text{ cell}/\mu\text{l}$ .

Progression to Stage 5 is usually determined on the basis of development of anergy. The fifth stage is known as AIDS related complex (ARC). In this phase the immune system becomes weak and the body becomes susceptible to other infections. In this opportunistic infection phase the antigen concentration in the blood increases exponentially. The T4 cell count falls less to  $200/\mu\text{l}$ .

Most people enter Stage 6 when the T4 cell count reduces to  $100/\mu\text{l}$  or less. This last stage is full blown AIDS. The replication of the virus flares up and the infection enters the final stage. The concentration of the antigen is very high and it slowly saturates towards the end. In the model, simulation results show that towards the end antigen has significantly decreased rates of multiplication. This aspect will be further explored in later versions. This last stage of the disease results in death.

In Figure 6.3, T4 helper cell concentration is plotted against time. In Stage 1, T4 cell concentration is almost normal. It stays constant around  $800\text{-}1000 \text{ cells}/\mu\text{l}$ . Within six months to a year chronic lymphadenopathy develops. The T4-cell count slowly declines. In Stage 2 the decline is faster. The cell count goes to  $400/\mu\text{l}$ . The declining rate is higher in Stage 3. The count falls below  $400/\mu\text{l}$ . In Stage 4 the cell count goes persistently lower. The cell count further reduces to  $200/\mu\text{l}$ . The cell count decreases to 100 in the fifth stage. Finally in Stage 6, full blown AIDS, there is hardly any trace of T4 cells. The antigen replicates wildly, killing the remaining T4 cells and hence any vestiges of the immune defense.



In Figure 6.4, monocytes and macrophages are plotted against time. Some monocytes and macrophages also carry CD4 receptors and they are attacked by HIV. They get infected and their number decreases with progression of AIDS. In the initial stages their number stays constant for a long time, but towards the terminating stage their number decreases.

Figure 6.5 is plotted for cytotoxic and natural killer cells. Cytotoxic cells have CD8 surface molecules. In a healthy person there are no cytotoxic cells. In case of infection the number of cytotoxic cells and natural killer cells increases in order to engulf the toxic antigen and rid the body of unfavorable substances. In this case initial concentration of cytotoxic cell is zero. When the person becomes infected the number of cytotoxic cells and natural killer cells increases. Functionally this is predicted in any infection. This fact is verified by clinical results that show increase in the CD8/CD4 cell ratio. The model comfortably simulates their production, circulation in blood in the second and third stage, and their reduction and destruction in the terminal stage.

The infected cell concentration are shown in Figure 6.6. The model simulates the conversion of healthy T4 cells into infected T-cells which malfunction due to the presence of antigen within them. The infected cells carry antigen receptor and combine with healthy T cells thus forming syncytia [6]. The number of infected cells increases with progression of the disease and the number of healthy cells decreases constantly.

Concentration of antibodies against this antigen is shown in Figure 6.7. Soon after acute HIV infection production of antibodies is stimulated. Their concentration rises in Stage 2 and 3. It stays high in Stage 4 and starts decreasing in Stage 5. In the final stage their number falls rapidly because the immune system depletes completely.

The three cases presented in this study (refer to Table 1) differ from each other in their initial concentration of the virus. The 3 cases are briefly discussed in Table 3, as shown below.

**Table 3.**

Stages	Period (days) in a particular stage		
	Case 1 (10 virus particles/ul)	Case 2 (50 virus particles/ul)	Case 3 (100 virus particles/ul)
Stage 0	0 - 560	0 - 370	0 - 295
Stage 1	560 - 640	370 - 480	295 - 400
Stage 2	640 - 700	480 - 530	400 - 485
Stage 3	700 - 730	530 - 565	485 - 515
Stage 4	730 - 760	565 - 595	515 - 545
Stage 5	760 - 810	595 - 650	545 - 610
Stage 6	810 - death	650 - death	610 - death

In Table 3 , you can see that as the dose of the initial concentration of the injected virus increases the patient enters the ascending stages of the disease faster. This survival time of the patient decreases as the dose is increased from 10 virus particles/ul to 100 virus particles/ul. Case 1 is the summation of the Figures 6.1 to 6.7 and it is plotted in Figure 6.8. This case was simulated again with the detox unit on. In the model this was incorporated by substituting values other than zero for  $k_{24}$ ,  $k_{25}$  and  $N_d$ . The result of this simulation case is shown in Figure 6.9. Comparing the cases with the machine off and machine on it can be easily seen that the concentrations of the antigen and infected cells are reduced considerably. More virus in the blood means more infectivity [13]. The initial virus concentration is increased for the second and third case. In the second case the virus concentration is increased to  $50/\mu\text{l}$ . Case 2 is shown in Figure 6.10. T cell concentration decreases more quickly and the survival time of the patient is decreased. This case is again simulated with the detox unit on as shown in Figure 6.11.

In the third case the virus concentration is increased to  $100/\mu\text{l}$ . Case 3 is shown in Figure 6.12. The patient manifests AIDS earlier than the patients of the first two cases. The third case is again simulated with the machine on (Figure 6.13) and it is seen again that the detox machine is powerful in decreasing the concentration of antigen and infected cells in the blood. The effect of the machine off and machine on for the 3 cases in shown in Table 4.

Table 4.

Stages	Period (days) in a particular stage					
	Case		Case 2		Case 3	
	machine off	machine on	machine off	machine on	machine off	machine off
Stage 0	0 - 560	0 - 565	0 - 370	0 - 390	0 - 295	0 - 300
Stage 1	560 - 640	565 - 645	370 - 480	390 - 495	295 - 400	300 - 420
Stage 2	640 - 700	645 - 705	480 - 530	495 - 555	400 - 485	420 - 495
Stage 3	700 - 730	705 - 735	530 - 565	555 - 585	485 - 515	495 - 530
Stage 4	730 - 760	735 - 765	565 - 595	585 - 615	515 - 545	530 - 565
Stage 5	760 - 810	765 - 825	595 - 650	615 - 675	545 - 610	565 - 630
Stage6	810 - death	825 - death	650 - death	675 - death	610 - death	630 - death

# INITIAL ANTIGEN BEHAVIOR

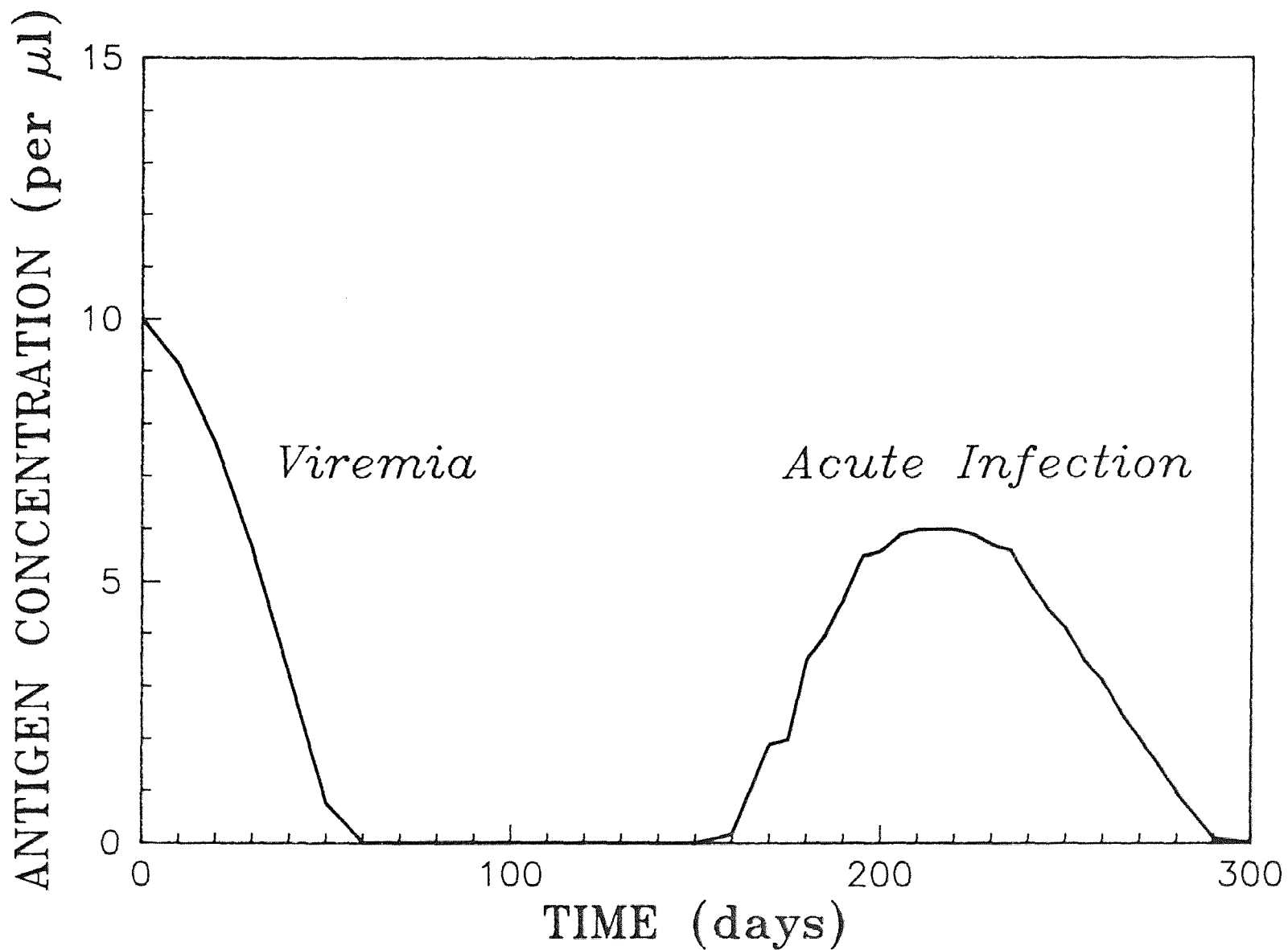


Figure 6.1

# ANTIGEN MULTIPLICATION

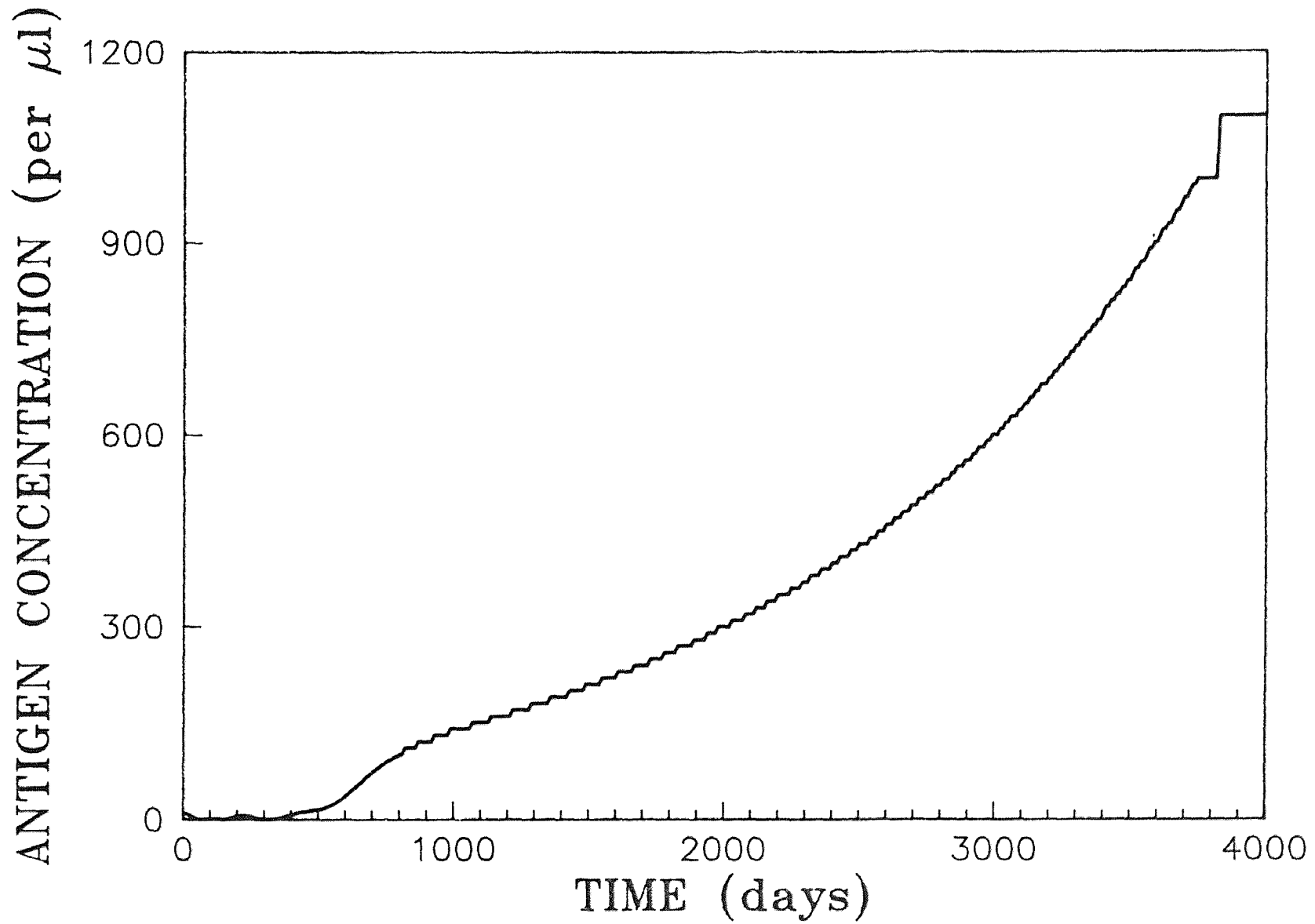
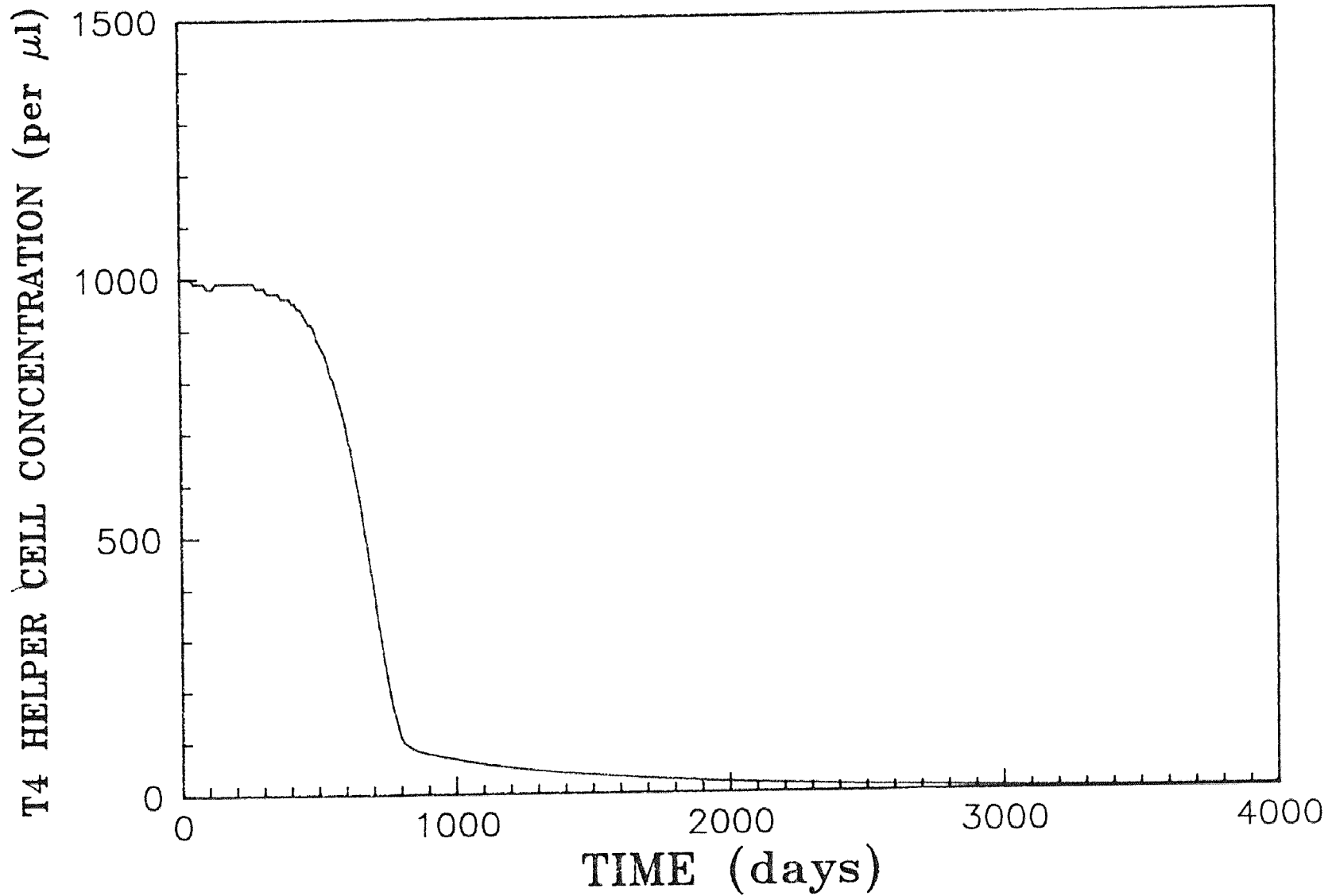


Figure 6.2

# DEPLETION OF T4 CELLS WITH PROGRESSION OF THE DISEASE



*Figure 6.3*

# SECONDARY IMMUNE CELLS

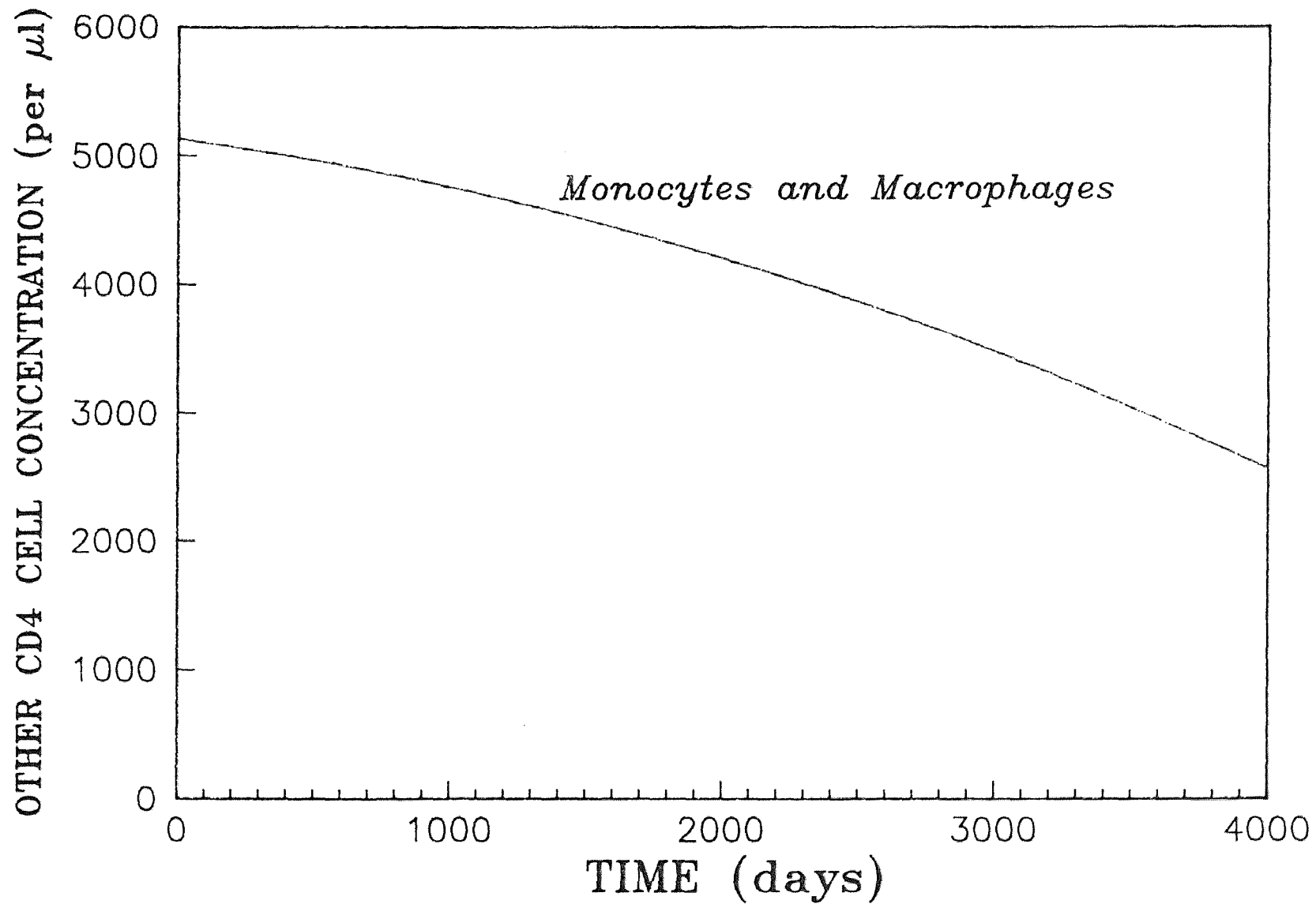


Figure 6.4

# CYTOLYTIC ACTIVITY

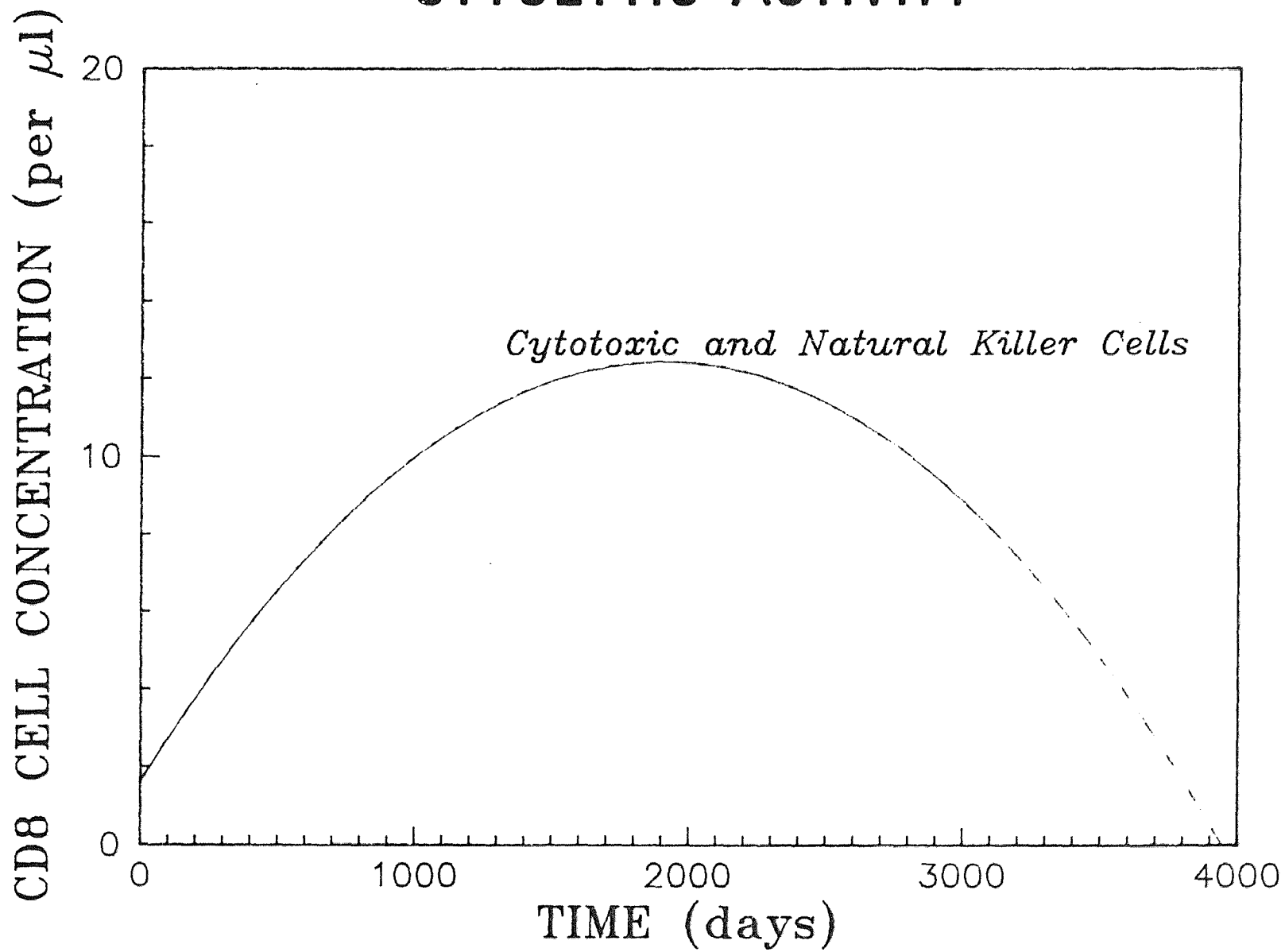


Figure 6.5

# INFECTED T CELLS AND SYNCYTIA

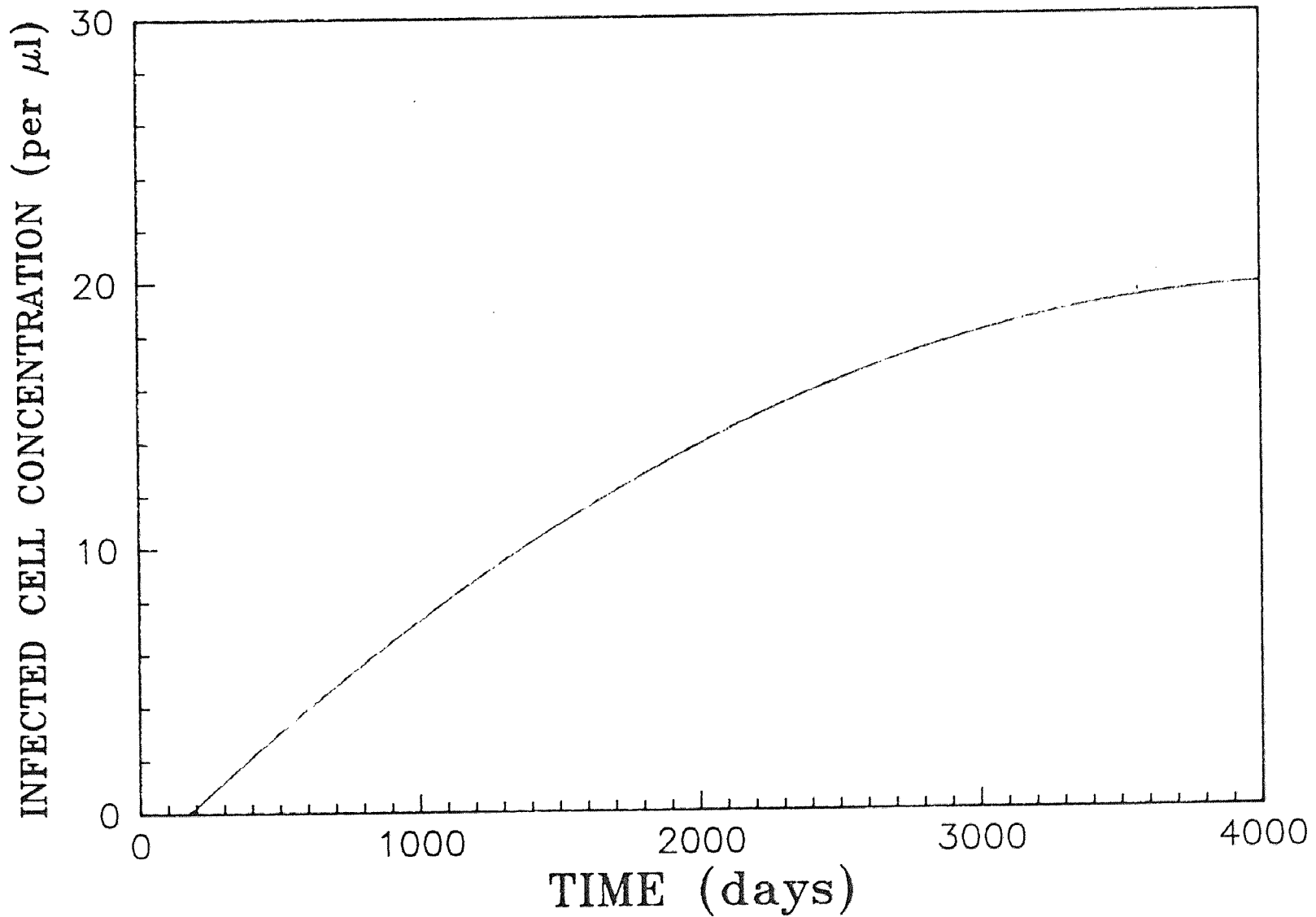


Figure 6.6



# HUMORAL ACTIVITY

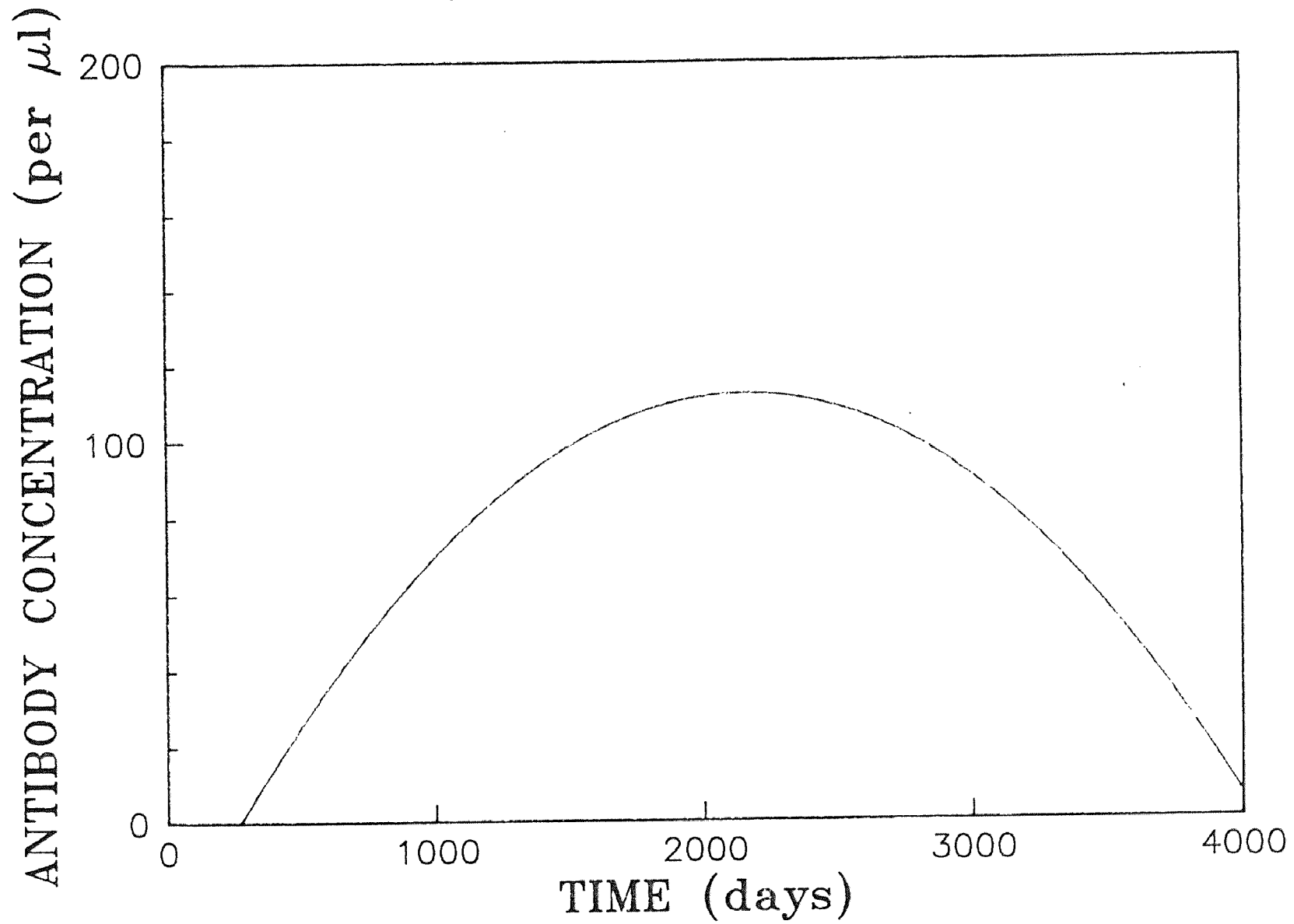


Figure 6.7

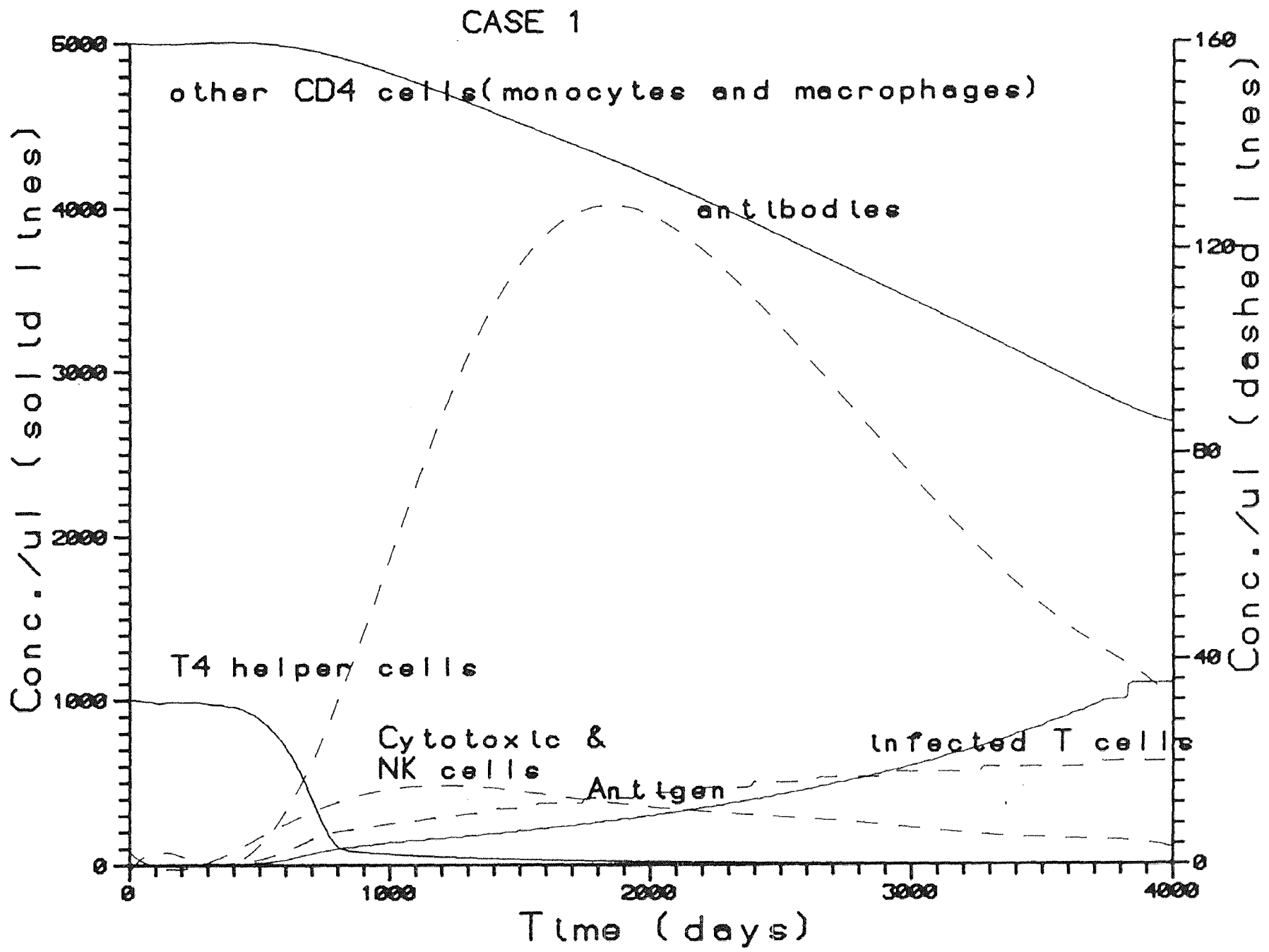


FIGURE 6.8

CASE 1 (Mech lne on)

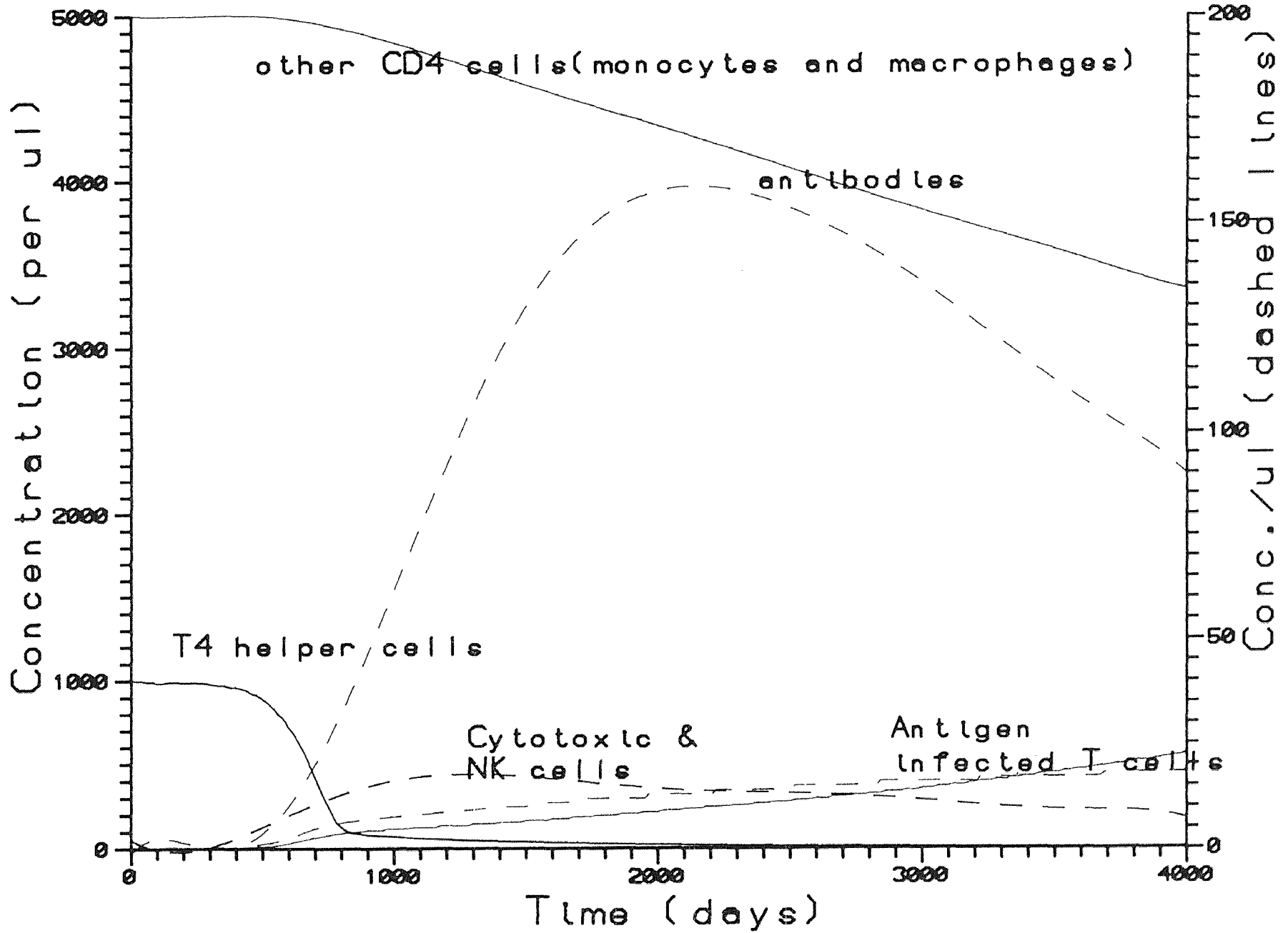


FIGURE 6.9

# CASE 2

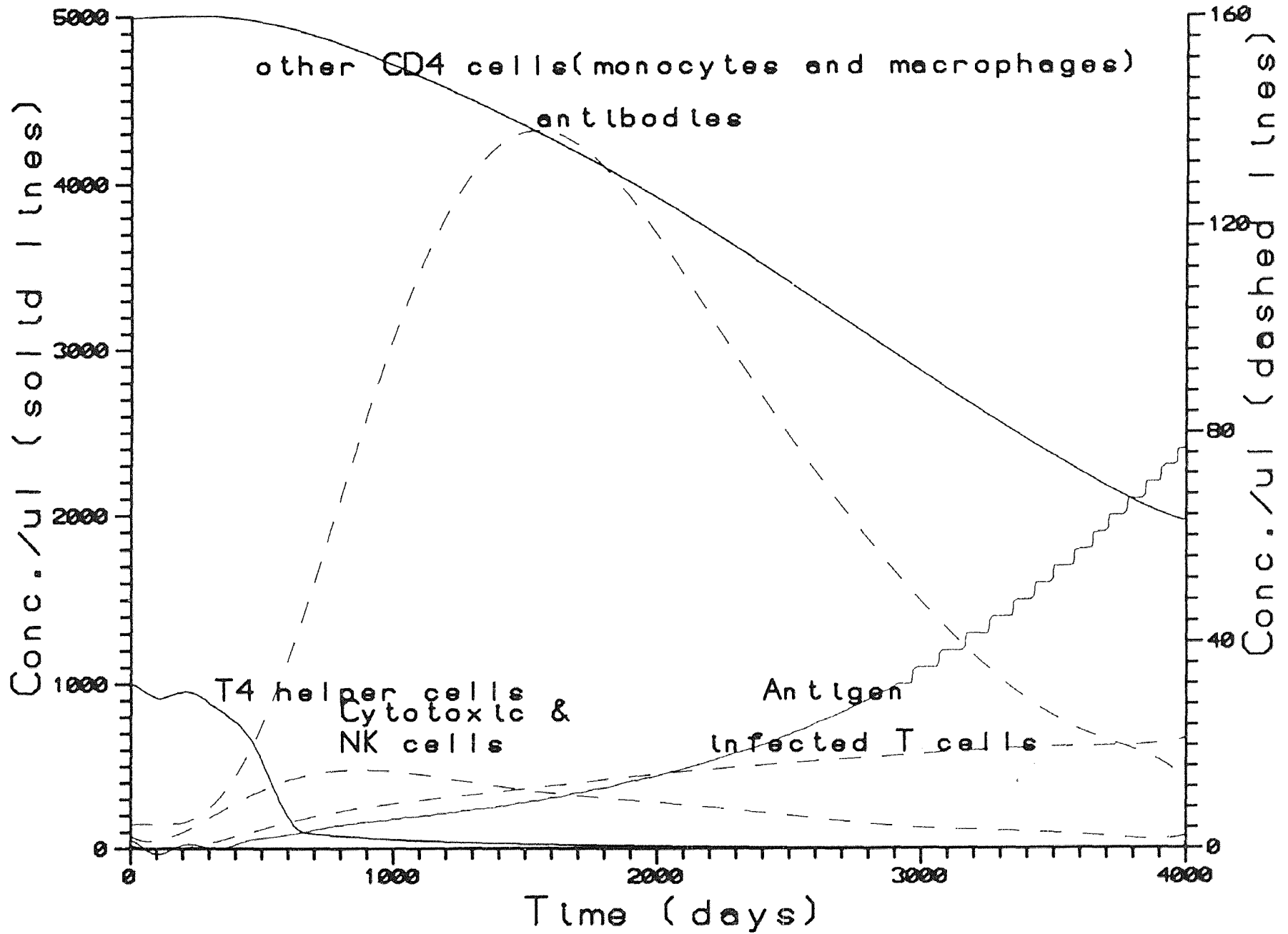


FIGURE 6.10

CASE 2 (Machine on)

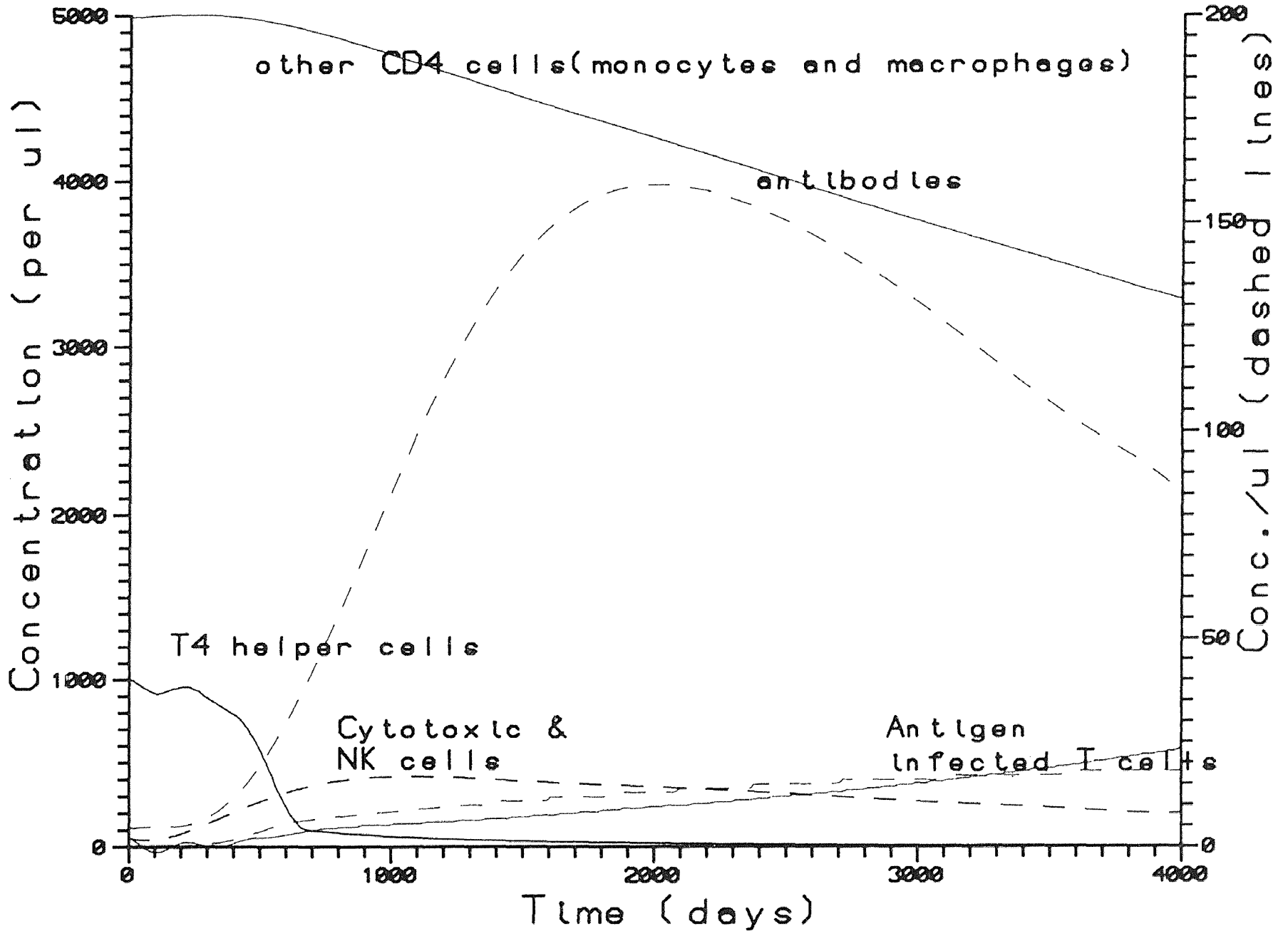


FIGURE 6-11

### CASE 3

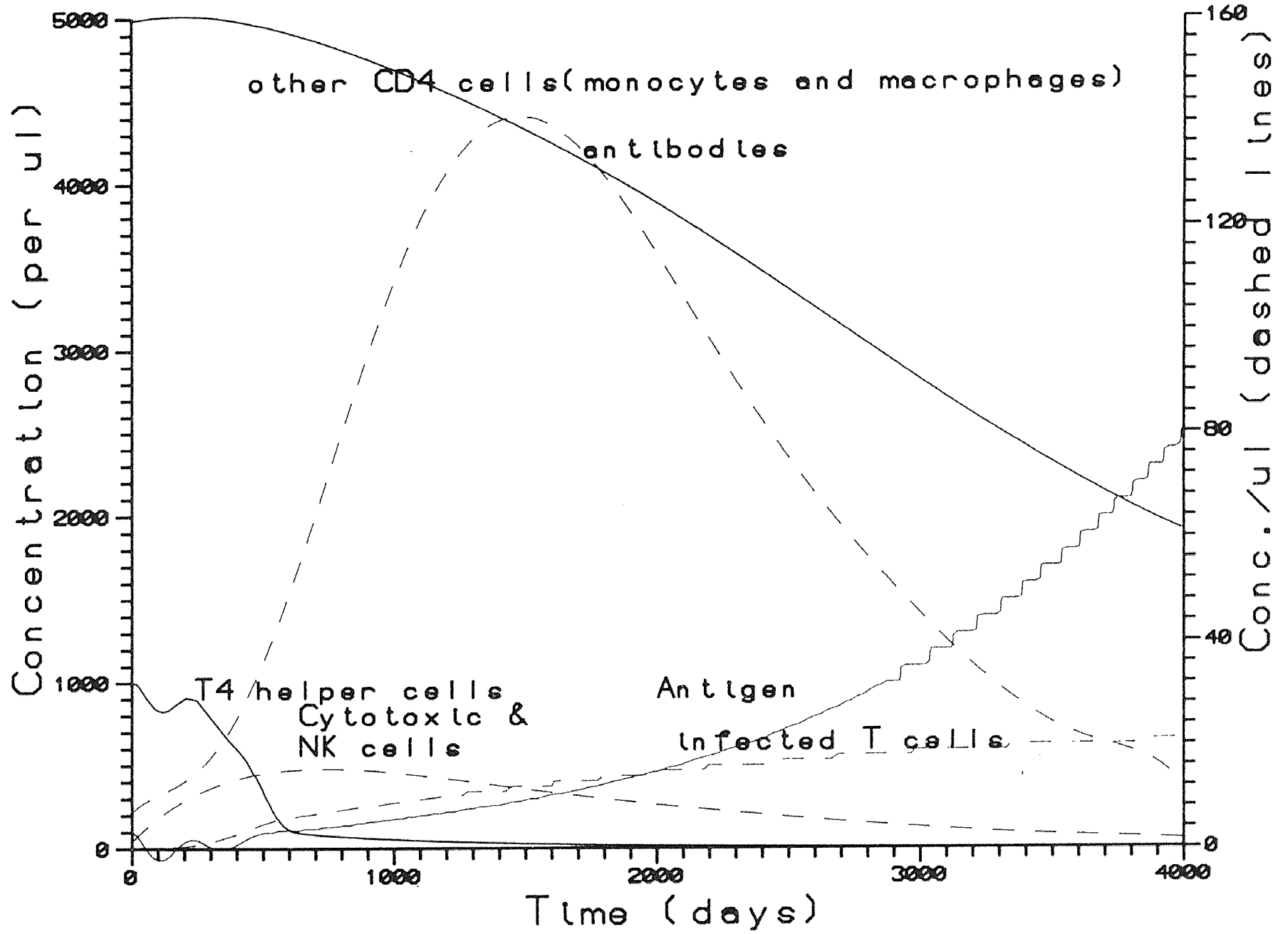


FIGURE 6-12

CASE 3 (Machine on)

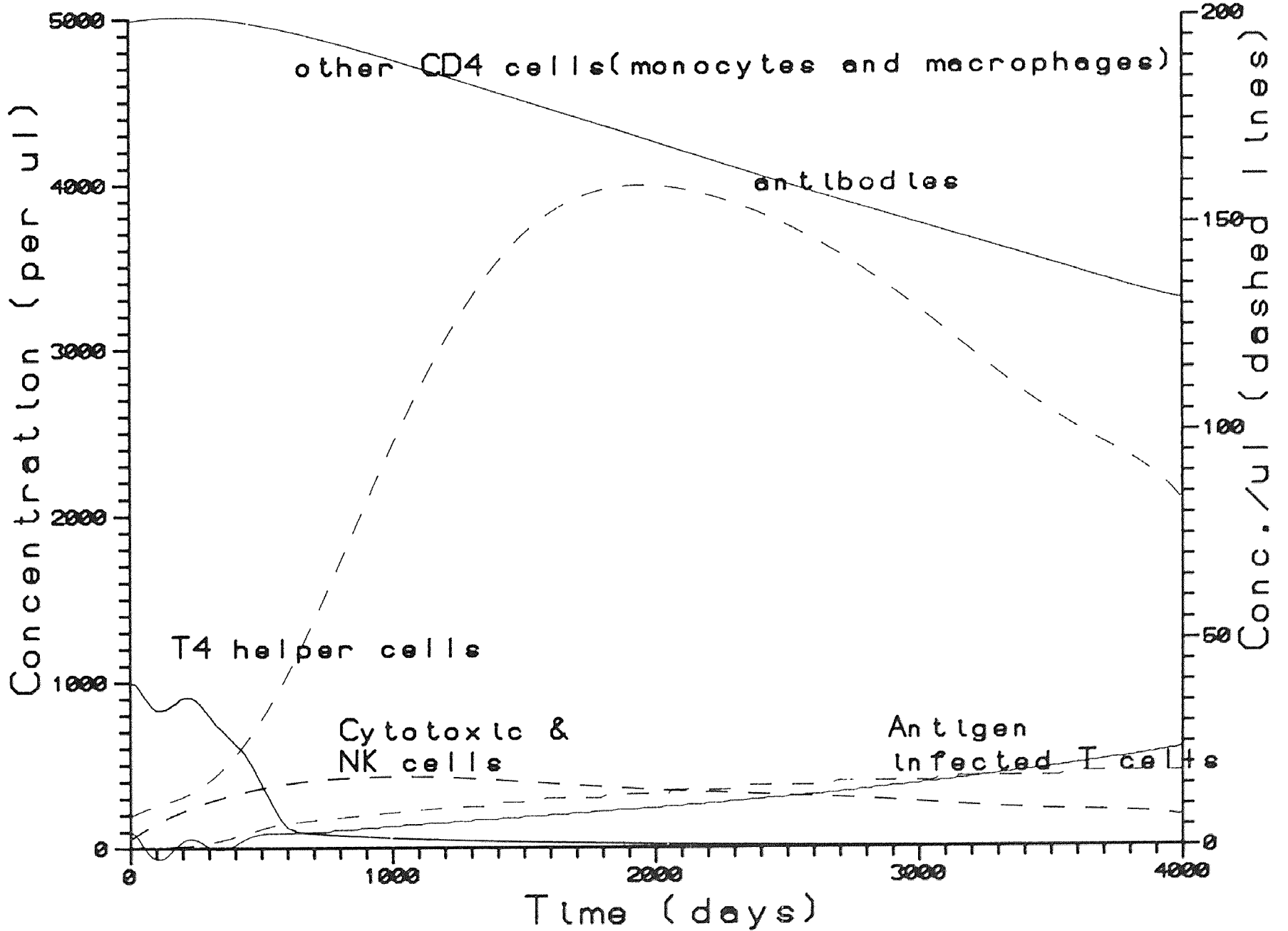


FIGURE 6.12.

# CHAPTER 7

## CONCLUSIONS

This model simulates HIV transmission, the subsequent prolonged period of acute infection during which the immune system suffers progressive decline and the acquisition of AIDS related terminal disease, and full blown AIDS. Following the infection contact, the individual enters a first stage during which viral antigen circulates unchecked. This asymptomatic stage varies, depending on the infective dose contracted by the individual.

After several weeks to several months, the individual develops antibodies to HIV, a process known as seroconversion [7]. During the period of acute infection, T4 helper cells which coordinate immune system responses are attacked by HIV and they are enlisted genetically to produce more virus. Consequently the infected individual's T4 cell count gradually decreases, rendering the individual susceptible to opportunistic diseases.

The three cases presented successfully simulate the progression of AIDS. The model-based investigations show that the theoretical results obtained from the simulation are in close agreement with clinical observations [19]. This model is flexible; addition of more variables to provide response details is easily accommodated.

The model can be readily extended to incorporate cytokine and chemotactic reactions with stimulation of different lymphokines. Antibodies to the core protein and the envelope protein can be simulated separately. B cells as well as plasma cells can be added in later versions of this model.



## Chapter 8

### Recommendation for further work.

Using this model we could not find the smallest dose of the initial concentration of injected antigen which would not result in AIDS manifestation. The search for this case can prove to be very useful in determining the threshold of HIV transmission and should be continued. Below this threshold the patient would not develop AIDS.

This simulation can be extended further by administration of anti-viral drugs to the patient. AZT which is the present drug used for many patients can be simulated. The dosage of the drug as well as the time of administration can be studied and incorporated in such a model.

Another research treatment for this disease is the injection of killer cells . The dose and the time of the killer cell injection can be simulated and studied.

In this model syncytia and infected cells were treated together. Further modeling along this track can be done by separately accounting for the infected T cells and syncytia. The detox unit can be simulated for the evaluation of syncytium removal vs infected cell removal.

In the experimental section, for the second stage separations we have not yet been able to find an accurate measure of the WBC/RBC ratio which gives a better separation. Experiments in recycling of blood are recommended.

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