MATHEMATICAL MODEL AND SIMULATION OF THE PROGRESSION OF CUTANEOUS T-CELL LYMPHOMA

by

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of the Progression of Cutaneous T-Cell Lymphoma.

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ABSTRACT

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A simple mathematical model and simulation of the progression of cancer with particular emphasis on Cutaneous T Cell Lymphoma was investigated. The mathematical model consists of a set of differential equations that first satisfies the homeostasis levels of the different components present and second predicts the dynamics during a diseased or unhealthy condition. The simulation was done using a FORTRAN program for integrating the differential equations.

Simulation of the immune system is important in evaluating the usefulness of particular sorts of biologically targeted therapies and in selecting the most promising treatment strategies for clinical trials. In particular, the effects of leukapheresis and photopheresis in the treatment of CTCL was investigated using the model and available data. Simulation is also important for testing the effectiveness of new drugs on the system.

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CHAPTER I

INTRODUCTION

Cancer arises as a result of genetic changes in previously normal body cells. These changes are induced by diet and nutrition, lifestyle (smoking, alcohol, etc), chemicals, and other environmental factors. The continued proliferation of a single abnormal cell results in cancer. Cancer cells do not divide themselves in an orderly or specific fashion, rather, they invade neighboring tissue and organs, forming a continuous mass of cells called a tumor. In their need for space the cells may metastacize, that is, they may travel to other parts of the body via the blood and lymph stream [1,2].

The immune system is a fairly intricate network of blood cells, chemicals and organs that protect the body against foreign organisms by recognizing potentially harmful invaders and destroying them. If any aspect of the system malfunctions because of poor nutrition, or is weakened or destroyed, that person may become susceptible to cancer and foreign microbial invaders [2].

The body defends itself from cancer cells by cell-mediated immunity. Cytotoxic T cells and/or natural killer (NK) cells travel to the location of abnormal cells, bearing on their surface antibodies to antigens of the kind that initiated the immune response, and directly kill the target cells via secreted chemicals without the intervention of other antibodies. The target cells are the body's own cells which have become genetically altered due to cancer and therefore, have membrane proteins which are different from those of normal body cells [3].

In general, developed countries have higher cancer incidence rates than less developed countries. This is probably a result of different lifestyles and environmental factors but a large element of it is undoubtedly due to an increased life-expectancy in the developed world. As is well known, cancer is more common in the elderly than in the young. Hence, the greater the success in reducing infant mortality and other causes of early death, the greater will be the number of people surviving to die of other causes, including cancer. There is also an association between occupational

exposure to carcinogens and cancer. In developed countries, cancer is second only to heart disease as the major cause of death [1].

Cutaneous T Cell Lymphoma (CTCL) is a malignancy that begins with helper T cells in the skin. In the U.S. there are between 7,500 and 10,000 new cases reported each year. Even though a recent increase in the incidence of the disease has been observed in patients under the age of 30, most CTCL cases are diagnosed in persons beyond the fourth decade of life. CTCL is more common in males than in females [4].

A simple mathematical model of the dynamics of CTCL will be developed in this project. The mathematical model will consist of a set of differential equations that first satisfies the homeostasis levels of the different components present and secondly predicts the dynamics during a diseased or unhealthy condition. The model of the immunology of CTCL is important in evaluating the usefulness of particular sorts of biologically targeted therapies and in selecting the most promising treatment strategies for clinical trials.

CHAPTER II

THE IMMUNE SYSTEM

The immune system is a diverse collection of cells found both in the blood and in organs and tissues throughout the body. White blood cells play a major role in the immune response. Lymphocytes, the most sophisticated of the cells involved in the immune response, are capable of very precise recognition of foreign molecules and cells. There are two classes of lymphocytes, T lymphocytes and B lymphocytes. Both types arise from stem cells in the bone marrow. The stem cells, however, lack the receptors that enable B and T cells to recognize specific molecules as targets for immune attack. Such immune receptors appear as the stem cells mature. As a result of maturation, each B or T cell ultimately comes to have many copies of one immune receptor, the antibody on its surface and is therefore able to recognize only one other molecule, the antigen [3].

Antigens are any molecules that can induce an immune response. The antigen may be an individual cell or it may be part of the surface of a body cell. Tumor cells contain molecules not present in normal cells that act as antigens. Antigens are presented by the macrophage to helper/inducer T cells which in turn stimulate other cells to attack the antigen. The cell also defends itself from antigens by synthesizing antibodies. Antibodies are synthesized and secreted by B cells in the presence of the antigen.

Macrophages are large white cells found in virtually all organs and tissues. They migrate between blood and tissue cells and gather at focal sites of infection where they engulf and kill foreign organisms. Macrophages may become highly cytotoxic when they become activated by lymphokines. The process begins when antigen-specific T cells become activated by the antigen, and they release lymphokines, which attract and activate macrophages. Activated macrophages are cytotoxic to the microorganisms and tumor cells. Resistance to a tumor is increased if the number of activated macrophages is increased. It is believed that macrophages do not phagocytize cancer cells, rather they may kill tumor cells by means of secreted products, such as lysosomal enzymes and oxygen metabolites [6].

Summary of roles of B, cytotoxic T, and helper T cells in immune responses. The two other less well understood lymphocyte populations are suppressor T cells, which inhibit B cells and cytotoxic T cells, and NK cells, which function similarly to cytotoxic T cells.

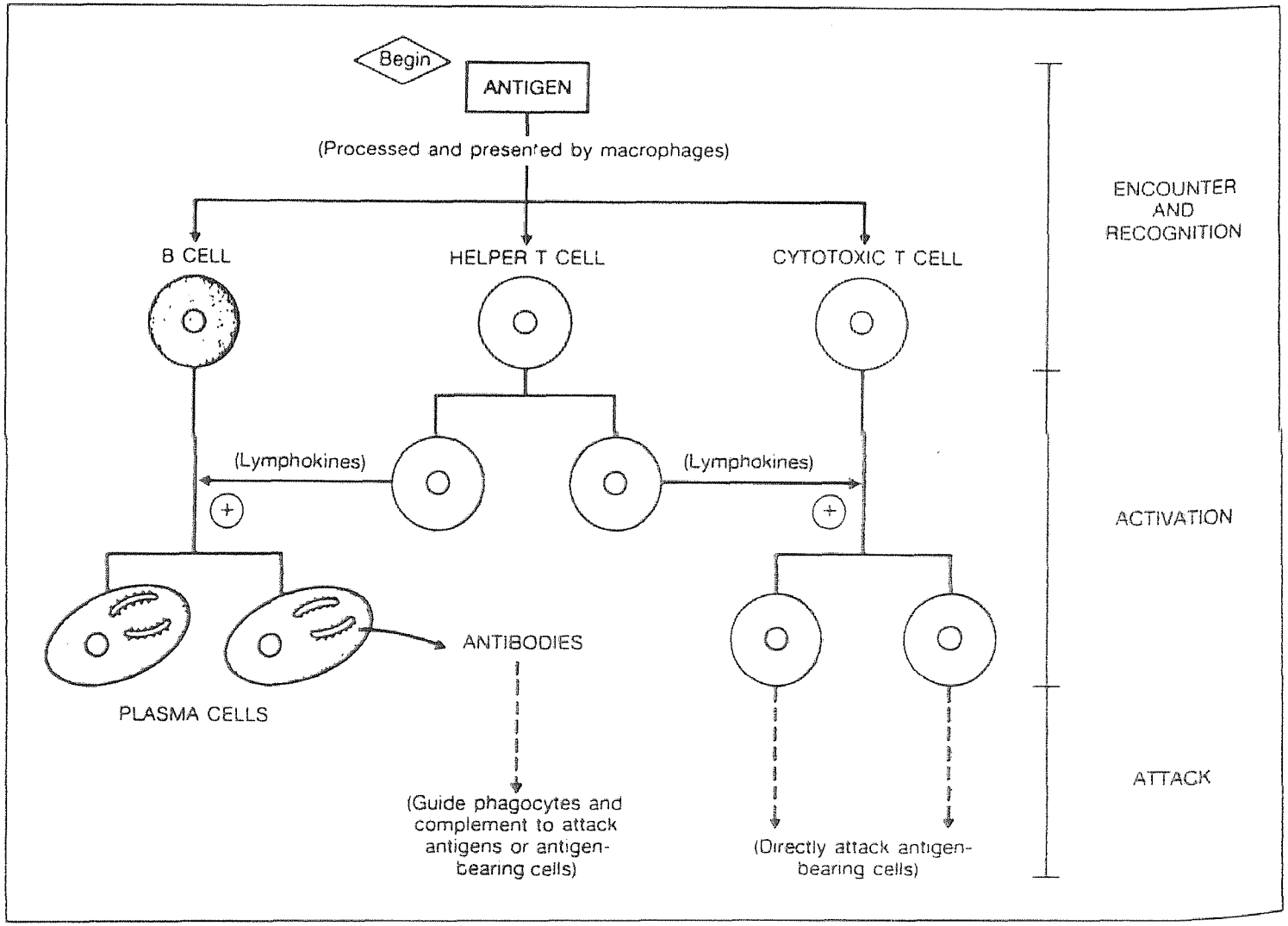
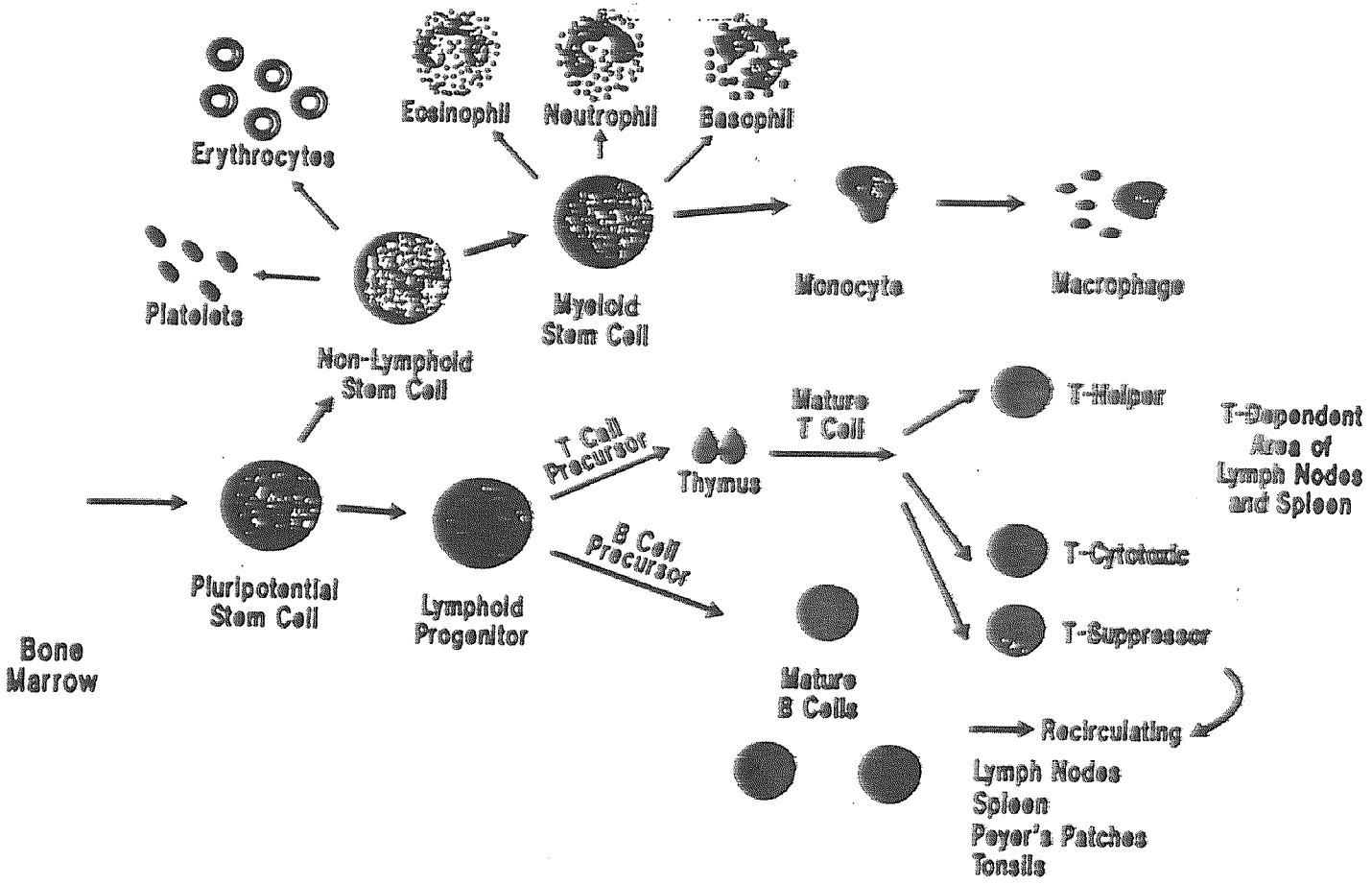


Figure 2.1 Taken from Human Physiology: The Mechanisms of Body Function. p.664



The developmental pathway of various cell types from a pluripotential bone marrow stem cell.

Figure 2.2. Taken from Immunology: A Short Course p.137

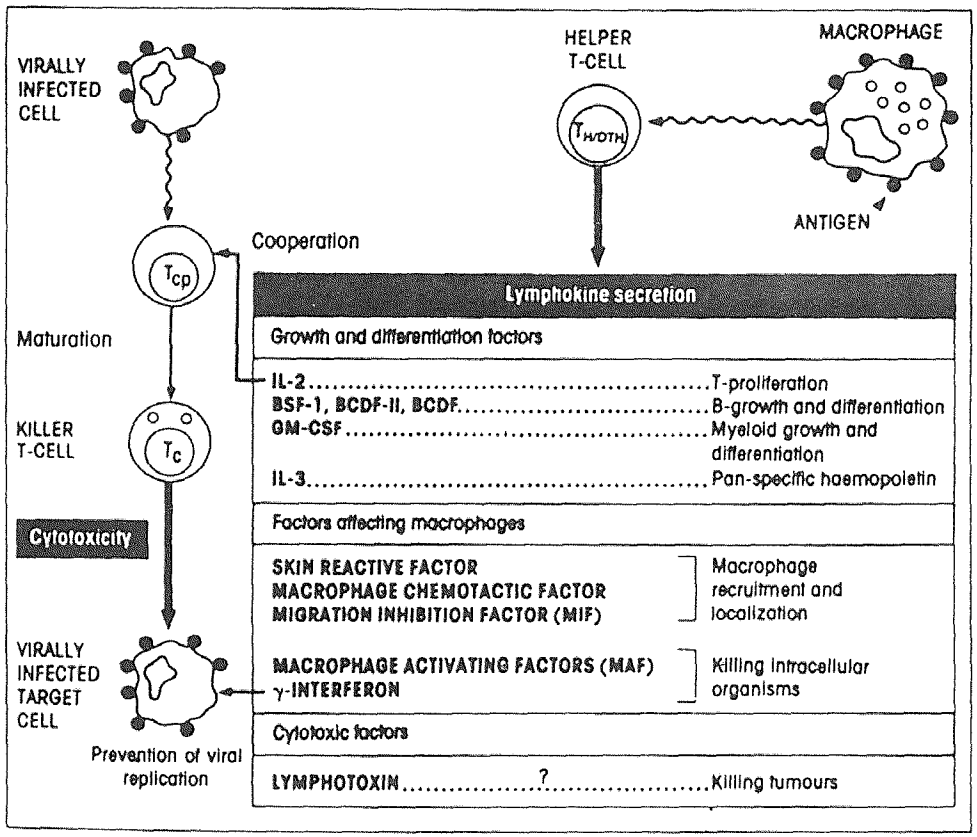
The identification of human lymphocyte subsets has been made relatively simple by the availability of a range of monoclonal antibodies that recognize cell surface antigens. These antigens are called CD1, CD2, CD3, and so forth. Mature T lymphocytes then split into two subpopulations. The helper/inducer population is CD4 positive and CD8 negative and accounts for about 60% of peripheral blood lymphocytes. The cytotoxic/suppressor population is CD4 negative and CD8 positive and accounts for about 25% of peripheral blood cells [7].

Functional T cells recognize the antigen by a specific cell surface receptor. Cytotoxic cells without T cell or B cell markers are natural killer (NK) and killer (K) cells. NK cells are lymphoid cells found in the spleen, lymph nodes, bone marrow and peripheral blood of humans. These cells are important in the host's defense against early stages of tumor growth, before the development of killer T cells and T cell-mediated activated macrophages. Killer cells attack tumor cells and cells infected by viruses and shoot them full of holes. They recognize target cells by passively absorbed antibodies. Helper T cells function in the secretion of lymphokines that activate B, cytotoxic T and NK cells and convert macrophages into effector macrophages [3,7].

The immune response can be divided into three major stages. The first stage involves the encounter and binding of antigens by lymphocytes that contain receptors specific for that antigen. The second stage involves the activation of the lymphocytes. Once the antigen is bound to the lymphocyte, the complex undergoes mitotic divisions producing several lymphocyte types that either attack the cell directly or activate attack cells to do so. The third stage is the attack stage. The activated lymphocytes attack antigens and antigen-bearing cells of the type that initiated the immune response. The nature of the attack depends upon the lymphocyte involved [3,8].

IMMUNOLOGICAL ASPECTS OF CANCER

Cell-mediated immunity is used by the body for protection from cancer cells. Cell-mediated responses are carried out by natural killer cells which directly destroy target cells without the intervention of antibodies. Natural killer cells, present in the cellular as well as the humoral system, seek out and destroy the body's own cells



The cell-mediated immune response. This operates through the generation of cytotoxic T-cells and the release of lymphokines through the stimulation of two distinct T-subpopulations, the cytotoxic cell precursor (T_{cp}) and the T-helper/inducer/delayed type hypersensitivity cells ($T_{h/dth}$). Different lymphokines may be produced by different lymphocyte subsets, and at this time it is not possible to say whether cells mediating delayed-type hypersensitivity (the skin manifestation of a cell-mediated immune response) represent a distinct subpopulation from the helpers. The intense proliferation induced by antigenic stimulation has not been shown but is essential for amplification of the response. Other lymphokines which activate osteoclasts and fibroblasts have been described.

Figure 2.3 Taken from Essential Immunology p.110

when they go wrong. They are directed at virally infected cells, antibody-coated cells, undifferentiated cells, and the cells from a number of different tumors. NK cells destroy malignant cells and virus-infected cells by direct cytotoxicity and the release of perforins in the absence of prior antigenic stimulation. NK cells produce interferon upon encountering target cells. The interferon then enhances NK activity by promoting the rapid differentiation of pre-NK cells. It also enhances T-cell and macrophage-mediated cytotoxicity [7].

Tumors induced by oncogenic viruses tend to gain new antigens that although distinct from virion antigens, are encoded by the virus and are referred to as tumor associated antigens (TAA) [7].

Chemically induced tumors differ from the virus-induced variety by carrying surface antigens unique to the tumor and not to the inducing chemical. Thus cells of a given tumor, arising from a single transformed cell, all share common antigens, while different tumors, even if induced by the same carcinogen, are antigenically distinct from one another [7].

If tumor cells are antigenically different from normal, they will be regarded as foreign and attacked. The major mechanism of tumor-cell destruction involves NK cells and cytotoxic T cells, although activated macrophages may also participate in this process [7].

The cytotoxic T or natural killer cell releases, by exocytosis, the contents of its secretory vesicles, perforin, into the extracellular space between itself and the target cell to which it is bound. The molecules of pore-forming protein secreted by the killer cell insert into the target-cell membrane. Once cells are exposed to perforin in the presence of calcium ions they are lysed within a few minutes because the monomeric molecules polymerize after entering the cell's membrane. The cell then becomes leaky and soon bursts [9].

Cytotoxic T cells are responsible for destruction of the tumor *in vivo*. Although helper T cells participate in the induction and regulation of cytotoxic T cells, the destruction of the tumor cell is achieved by the cytotoxic T lymphocytes (CTL) with specificity for the antigens on the surface of the tumor cell. The main target of the cytotoxic T lymphocyte are virus-induced tumors. T cells recognize and bind to an antigen only when it is presented by a protein molecule of the body's own major his-

tocompatibility complex (MHC). This MHC restriction explains why cytotoxic T cells only recognize the body's own cells such as cancer cells and virus-infected cells. Once either the killer T cell or the natural killer cell has identified its target, the killer cell binds tightly to the target cell. This close contact triggers the lethal process and also ensures that neighboring cells are not indiscriminately destroyed [3,9].

There are several factors that may influence the escape of tumor cells from destruction. First, the tumor may not provide sufficient stimulus to induce an immune response until it has reached a size at which it cannot be controlled by the host. Second, tumor cells that are different from the host cells will be rapidly identified and eliminated without leading to disease. Those tumors that do develop must, therefore, be selected for their inability to stimulate the host's immune response. It is also possible that the tumor may be in an area of the body, such as parts of the eye or in tissues of the central nervous system, that are inaccessible to effector cells of the immune response or their products. Finally, tumor-specific suppressor T cells may decrease the immune response [7,10].

Generally, as a malignancy progresses more abnormal and aggressive subclones develop, which ultimately overwhelm the host [7].

Tumors occur more frequently in immunosuppressed individuals than in normal individuals. Patients subjected to radiation or immunosuppressive drugs, show a much higher incidence of lymphoid tumors. Older individuals are also more prone to a wide range of tumors because their immune response is strongly reduced [1].

CHAPTER III

CARCINOGENIC PROCESS

Environmental factors are thought to play a prominent role in the etiology of cancer. Of these environmental factors, chemical agents, naturally occurring or synthetic, are believed to be a major etiological component [11].

We are all exposed to carcinogens every day, however, not all people exposed to a carcinogen have the same probability of getting cancer. This is due to the fact that enzymes can break down or activate the carcinogen at different speeds in different people to either render it harmless or promote it to cause cancer. The enzyme's activity is, in part, genetically dependent. They will destroy or activate carcinogens to varying degrees according to inherited tendencies. People with certain inherited diseases and the immunodeficiency syndromes, are more prone to getting cancer [2].

Most carcinogens are chemical. Carcinogens derived from car emmisions, industrial activity, and burning of solid wastes and fuel remain in the air from 4 to 40 days and travel long distances. Our drinking water also contains a number of carcinogens, including asbestos, arsenic metals and synthetic organic compounds. Food may contain carcinogens either as natural components of the food or in the form of additives. Carcinogens may be present in food that is spoiled (by bacteria, fungus, or chemicals), improperly washed, or contaminated with industrial pollutants [2].

The effects of carcinogens become more prominent when nutritional deficiencies exist. These deficiencies can directly cause damage to genetic material and then repair it abnormally, which may lead to the development of cancer. In diets moderately deficient in protein, phagocytes and T cells are reduced in number, and their ability to kill cancerous and other abnormal cells is impaired [2].

Chemical carcinogens are a highly diverse collection of chemical substances, including organic and inorganic chemicals, solid-state materials, hormones and immunosuppressants. The carcinogenic process in which normal cells are converted to malignant ones, involves two steps. First, normal cells are converted to neoplastic cells. Carcinogens cannot initiate the process leading to cancer directly. They undergo enzymatic activation in the body and form a reactive ultimate carcinogen, or in-

itiator. Initiators bind covalently or by hydrogen bonds with a variety of cellular macromolecules, including DNA. Although DNA can be repaired by repair enzyme systems, if the cell replicates while the DNA is damaged, permanent mutational changes in the cell's genetic material may be produced. These alterations generate a permanently abnormal cell with an altered genotype and distinct phenotypes. The second step of the carcinogenic process involves the proliferation of abnormal cells beyond tissue constraints to form tumors. It is only when initiators become exposed to promoters, such as during wound healing and regenerative growth, that the cells become "irreversibly malignant" [11,12].

Physical carcinogens are introduced into the body in the form of ultraviolet light or by x-radiation. Ionizing radiation produces radicals and electrons which react to yield many different kinds of free radicals. Free radicals are high energy, unstable atoms possessing an extra electron that transfer energy to nearby substances in order to become stable. When radicals are made in the body, the high energy is transferred to body tissues and causes extreme damage that may lead to the development of cancer. Ultraviolet light forms free radicals in the body which inhibit genetic material (DNA) synthesis, protein synthesis, interrupt normal cell replication, and causes skin cancer. A mild sunburn decreases the function of circulating lymphocytes for as long as 24 hours [2].

CHAPTER IV

CUTANEOUS T- CELL LYMPHOMA (CTCL)

Cutaneous T-Cell Lymphoma (CTCL) is a neoplasm of helper T cells that infiltrate the skin and have an affinity for the T cell zone of lymph nodes and the spleen [15]. The common designation CTCL was given in 1975 to Sezary syndrome, mycosis fungoides, the nonleukemic process, other clinical and histopathologic cutaneous lymphomas often described as reticulum-cell sarcoma, lymphoma cutis, and histiocytic lymphoma, and most other adult T-cell leukemias [4].

Exposure to environmental toxins of industrial origin such as petrochemicals, metals, solvents and pesticides appears to contribute to and to increase the chances for development of CTCL. These chemical carcinogens trigger the phenotypic expression of uncontrolled proliferation [4].

CTCL has both a cutaneous phase and a leukemic phase. Clinically CTCL is confined to the skin for a long period of time with the development of scaly, eczematous, or erythematous patches followed by infiltrated lichenified plaques, and progressing to tumors [13]. At the tumor stage, the neoplastic cells become highly malignant and lose their affinity for the skin. This is probably due to the presence of new malignant clones of T lymphocytes that have lost their affinity for the skin. The leukemic phase of CTCL involves widespread skin and visceral organ involvement [4]. These patients usually have erythroderma and a large number of malignant lymphocytes in the peripheral circulation. There is only very limited bone marrow infiltration in patients with CTCL. The extent of the disease in the skin, lymph nodes, and visceral organs, as well as the number of circulating abnormal lymphocytes determines the patient's prognosis and survival time. Involvement of lymph nodes and spread into peripheral blood and visceral organs usually results in the patient's death due to organ dysfunction and/or infectious complications.

The malignant T cells initially have the characteristics of normal mature helper T cells, demonstrating CD3 and CD4 positivity, CD8 negativity, and affinity for the epidermis. In advanced stages, the malignant T cells express altered surface antigens, and lose their affinity for the skin [14].

There is a substantial increase in the total number of peripheral lymphocytes as malignant helper T cells proliferate uncontrollably in CTCL patients. Studies performed by Kung, et al claim that lymphocytes are increased 6-200 fold [15]. Skin testing reveals an impaired cell-mediated immunity. Although there is a marked reduction in the number of circulating B cells, normal or elevated levels of immunoglobin production are maintained. This is probably due to the fact that the residual circulating normal B cells may be hyperstimulated by the large number of neoplastic T cells with helper activity [16]. The neoplastic lymphocytes act as helper T cells but not suppressor T cells in immunoglobin production.

Various second malignancies have been reported in patients with CTCL. It is possible that the use of immunosuppressive drugs and radiotherapy in the treatment of a malignancy may influence the development of a second neoplasm. Also, the patient may be genetically predisposed on the basis of a family history of cancer. Of special interest is the development of B cell neoplasms in patients with CTCL. Stimulation of B cells by neoplastic helper/inducer T lymphocytes may potentiate the appearance of the second neoplasm [17].

In advanced stages of the disease the abnormal cells make up a large percentage of the total body white blood cells. There are two possible reasons for the clinically observed suppression of T-cell mediated immunity in patients with cutaneous T-cell lymphoma. First, normal T cells capable of responding to foreign antigens are diluted out in the peripheral blood by nonreactive neoplastic T cells. Since malignant T cells are incapable of specific response to microbial antigens patients may become severely immunocompromised. Second, the neoplastic T cells produce macrophage inhibitory factor which interferes with monocyte mobilization to peripheral sites and suppresses the ability of the host to express delayed hypersensitivity, further compromising the immunologic defenses of the patient [18]. Patients with CTCL are prone to various second malignancies.

CHAPTER V

MATHEMATICAL MODEL

The present model consists of several parameters and equations which describe the progression of CTCL in a patient. The model is set up in such a way that it takes into account the multiplication and destruction of the white blood cell population. White blood cells are destroyed when they bind with malignant cells and due to normal cell aging.

Carcinogens alter the normal functioning of DNA molecules, causing mutations. As the abnormal cells proliferate, changes in the phenotypic properties become apparent and spread to the new cells.

Normal T cells become abnormal at a rate dependent on the amount of carcinogen present in the patient's environment and on the body's ability to break down the carcinogen and render it harmless.

Malignant T cells proliferate at a certain rate in the patient. The white blood cells attack the malignant T cells and destroy them. Nonetheless, they are not able to keep up with the rapid multiplication of malignant cells. Eventually healthy immune cells capable of responding to foreign antigens are diluted by neoplastic T cells and hence there is a breakdown of cell-mediated immunity.

One potential treatment for CTCL is the combined use of the anti-twister extracorporeal detoxification system (detox unit) available at NJIT Biotechnology Laboratory with the photopheresis treatment being studied by Armus, et al. [13] Photopheresis involves the collection of WBC rich blood by extracorporeal centrifugation after the patient has taken methoxsalen (8-MOP). The leukocyte-rich portion of the blood is then extracorporeally exposed to UVA light and returned to the patient. Exposure to UVA apparently activated 8-MOP which then destroys malignant cells.

Photopheresis is considered mathematically in this model as the targeted destruction of methoxsalen-weakened malignant T cells by ultraviolet radiation.

Leukapheresis is also included mathematically as the removal of malignant cells, along with destruction of various relative amounts of healthy T cells, depending on machine efficiency.

From the above discussion the critical relations can be tabulated as follows.

Table 5.1

System of Equations		
Eqn No.		Description
1	$T_n + C \rightarrow$ abnormal cell $\frac{d[T_n]}{dt} = -k_1[T_n][C]$	Healthy T cells may become abnormal when they are exposed to carcinogens.
2	$T_m \rightarrow T_m + T_m$ $\frac{d[T_m]}{dt} = k_3[T_m]$	This shows the proliferation of malignant helper T cells.
3	$T_m \rightarrow M$ $\frac{d[M]}{dt} = k_4[T_m]$	The presence of malignant helper T cells causes normal helper T cells to release lymphokines that activate macrophages.
4	$T_m \rightarrow T_c$ $\frac{d[T_c]}{dt} = k_5[T_m]$	The presence of malignant T cells causes normal helper cells to stimulate the production of cytotoxic cells, natural killer cells and antibodies that recognize malignant cells and try to destroy them.
5	$T_m \rightarrow$ antibodies $\frac{d[Ab]}{dt} = k_6[T_m]$	In the presence of malignant cells, normal helper T cells hyperstimulate B cells to produce the antibodies.
6	$M + T_m \rightarrow death$ $\frac{d[T_m]}{dt} = -k_7[M][T_m]$	A macrophage destroys a malignant T cell. We have assumed the destruction of malignant T cells and macrophages after binding together.
7	$T_c + T_m \rightarrow death$ $\frac{d[T_m]}{dt} = -k_8[T_c][T_m]$	Cytotoxic and NK cells destroy a malignant cell. We have assumed the destruction of malignant T cells and cytotoxic and NK cells after binding together.

8	$Ab + T_m \rightarrow death$ $\frac{d[T_m]}{dt} = -k_9[Ab][T_m]$	We have assumed the destruction of malignant helper T cells and antibodies after binding together.
9	$T_c \rightarrow inactivation$	Cytotoxic cells become inactive due to the suppression of cell-mediated immunity.
10	$Ab \rightarrow inactivation$	Antibodies may become destroyed in the blood after a given period.
11	$M \rightarrow inactivation$	Macrophage amounts may also be reduced due to the production of macrophage inhibitory factor.
12	carcinogen $\rightarrow$ *destruction* $\frac{d[C]}{dt} = -k_{14}[T_n][C]$	The carcinogen becomes metabolized by the cell after it is introduced.
13	$T_m \rightarrow$ *destruction/removal* $\frac{d[T_m]}{dt} = -k_{20}[T_m]$	Malignant T cells may be destroyed or removed by leukapheresis.
14	$T_n \rightarrow destruction$ $\frac{d[T_n]}{dt} = -k_{21}[T_n]$	Normal T cells may be destroyed due to inefficiencies in treatment by leukapheresis on the detox unit

SYSTEM OF EQUATIONS

Change in normal helper T cell concentration with respect to time:

The rate of change of the concentration of normal helper T cells with respect to time is controlled by four terms. The first and second terms are the production and destruction respectively of T helper cells in the blood. The cells are produced at a certain rate in the blood and are destroyed due to cell aging. The reasoning behind these rate terms is borrowed from chemical kinetics and steady state assumptions used in petrochemical technology. They were included to numerically impose homeostasis on the system. The third term provides a decrease in the concentration due to the transformation of normal T cells into abnormal cells as they come into contact with carcinogens (Equation 1). An adjustment term k15, was included to keep the normal cell concentration from going back to homeostasis when the car-

cinogen concentration goes to zero as it becomes integrated into the cells DNA. When the detox machine is turned on for leukapheresis treatment there is a destruction of healthy T cells due to inefficiencies (Equation 14).

$$\frac{d[T_n]}{dt} = \frac{k_{10}}{k_{11} + [T_n]} - \frac{k_{12}[T_n]}{k_{13} + [T_n]} - k_1[T_n][C] - k_{15} - k_{21}[T_n]$$

Change in malignant helper T cell concentration with respect to time:

The concentration of malignant helper T cells increases as abnormal cells become malignant and as these malignant cells proliferate (Equation 2). The malignant T helper cell concentration decreases when the cytotoxic cells, natural killer cells, macrophages or antibodies recognize malignant cells and try to destroy them (Equations 6-8). Concentration is also decreased as malignant T cells are decreased or removed by leukapheresis on the detox unit (Equation 12).

$$\frac{d[T_m]}{dt} = k_1[T_n][C] + k_3[T_m] - k_7[M][T_m] - k_8[T_c][T_m] - k_9[Ab][T_m] - k_{20}[T_m]$$

Change in cytotoxic and natural killer cell concentration with respect to time:

Production of cytotoxic and natural killer cells is stimulated by normal T helper cells in the presence of malignant cells (Equation 4). Their concentration decreases as the cells attack the malignant cells (Equation 8).

$$\frac{d[T_c]}{dt} = k_5[T_m] - k_8[T_c][T_m]$$

Change in macrophage concentration with respect to time:

The first and third terms are, once again, production and destruction rates included to numerically impose homeostasis on the system. Macrophages are activated by lymphokines secreted by normal helper T cells in the presence of

malignant T helper cells (Equation 3). Their concentration decreases as they attack the malignant cells (Equation 6).

$$\frac{d[M]}{dt} = \frac{k_{19}}{k_{16} + [M]} + k_4[T_m] - \frac{k_{17}[M]}{k_{18} + [M]} - k_7[M][T_m]$$

Change in antibody concentration with respect to time:

Antibody production is stimulated by the presence of malignant cells (Equation 5). Their concentration decreases as they attack the malignant cells (Equation 8).

$$\frac{d[Ab]}{dt} = k_6[T_m] - k_9[Ab][T_m]$$

Change in carcinogen concentration with respect to time:

Carcinogen concentration decreases as it becomes metabolized in the body. Once these are metabolized they act like free radicals that will form malignancy (Equation 12).

$$\frac{d[C]}{dt} = -k_{14}[T_n][C]$$

where:

C carcinogen concentration
Tm malignant CD4 + helper cell concentration
Tn normal CD4 + helper cell concentration
M Macrophage concentration
Tt total CD4 + helper T cell concentration
Tc cytotoxic and natural killer cells concentration
Ab antibody concentration
k1 rate constant for abnormal cell formation
k2 rate constant at which abnormal cells become malignant
k3 rate constant for malignant T helper cell multiplication
k4 rate constant for normal production of macrophages
k5 rate constant for production of cytotoxic and NK cells stimulated by malignant T cells
k6 rate constant for production of antibodies stimulated by malignant T cells
k7 rate constant for the destruction of macrophages and malig nant T cells after

binding
k8 rate constant for the destruction of cytotoxic cells and malignant T cells after binding
k9 rate constant for the destruction of antibodies and malignant T cells after binding
k10 rate constant for normal production of CD4 + cells
k11 rate constant for regulation of CD4 + cell production
k12 rate constant for normal destruction of CD4 + cells
k13 rate constant for regulation of CD4 + cell destruction
k14 rate constant for metabolization of carcinogen
k15 adjustment term added to maintain a decrease in the number of normal T cells as they become malignant, otherwise they would go back to homeostasis.
k16 rate constant for the regulation of macrophage production
k17 rate constant for the normal destruction of macrophages
k18 rate constant for regulation of macrophage destruction
k19 rate constant for normal production of macrophages
k20 rate constant for the removal of malignant T cells by the detox unit
k21 rate constant for the unwanted removal of normal T cells along with malignant T cells.

CHAPTER VI

ESTIMATION OF MODEL PARAMETERS

Clinical data for the concentration of T helper cells was taken from immunological work done previously by Anju Nagpal of this lab [19]. The normal count of helper T cells is 1000/ul and the cytotoxic cell concentration is 750/ul (15% of combined monocyte and polymorph count of 5000/ul). The antibodies to the virus are zero for a non-diseased individual.

Healthy individuals have a normal blood cell count. For this reason we formulated our equations such that the rate of production of healthy cells was equal to the rate of destruction at homeostasis. The true rates would be patient specific but we have assumed certain values for the rate constants (Table 6.1) and kept them constant.

The results for homeostasis are shown in Figure 6.1.

Figure 6.2 shows that a perturbation of the system, such as by white cell removal, leads to recovery and homeostasis. The number of cells may be decreased from normal due to infection; however, our immune system model can "fight" the depletion and return the cell levels to normal after some time. The amount of time required is dependent on the rate constants which in turn are patient specific. For our simulation in Figure 6.2, we decreased the number of helper/suppressor T cells and the number of macrophages to various levels and in all cases there was a return to the homeostasis level. Note that recovery from homeostasis does not take place for five years - too long to be reasonable. This needs to be modified in future models.

The remaining rate constants were arbitrarily determined through knowledge of how the immune system works. The rate constants are patient specific and can be determined for a particular patient by taking WBC counts periodically.

Deterioration in the patient's condition should also be considered when calculating the rate constants for the equations. That is, even within the same patients, the constants change from time to time, depending upon the patient's state of health.

Once the rate constants are known, the patient-specific model can be simulated. This will help to determine patient survival time and the method of treatment that will be most appropriate for the patient. A patient-customized model may be instrumental in pharmakokinetics to determine the optimum dosage of drug for the patient. The model may also aid in determining the best form and dosage of radiation treatment. Ideally, maximum damage to tumor cells and minimum damage to normal cells is desired.

Table 6.1

MODEL PARAMETERS		
Base Case Values	**Rate Constants**	
Normal helper T cells = 1000/*ul*	$k_1 = 0.004$	$k_{12} = 1.9048$
Malignant helper T cells = 0.0/*ul*	$k_2 = 0.0$	$k_{13} = 1000$
Cytotoxic T and NK cells = 750/*ul*	$k_3 = 0.004$	$k_{14} = 0.000004$
Macrophages = 100/*ul*	$k_4 = 0.00005$	$k_{15} = 0.1$
Antibodies = 0.0/*ul*	$k_5 = 0.0001$	$k_{16} = 50$
Carcinogen = 1/*ul*	$k_6 = 0.003$	$k_{17} = 4$
	$k_7 = 0.0000000001$	$k_{18} = 100$
	$k_8 = 0.0000001$	$k_{19} = 300$
	$k_9 = 0.000001$	$k_{20} = 0.0$
	$k_{10} = 1000$	$k_{21} = 0.0$
	$k_{11} = 50$	

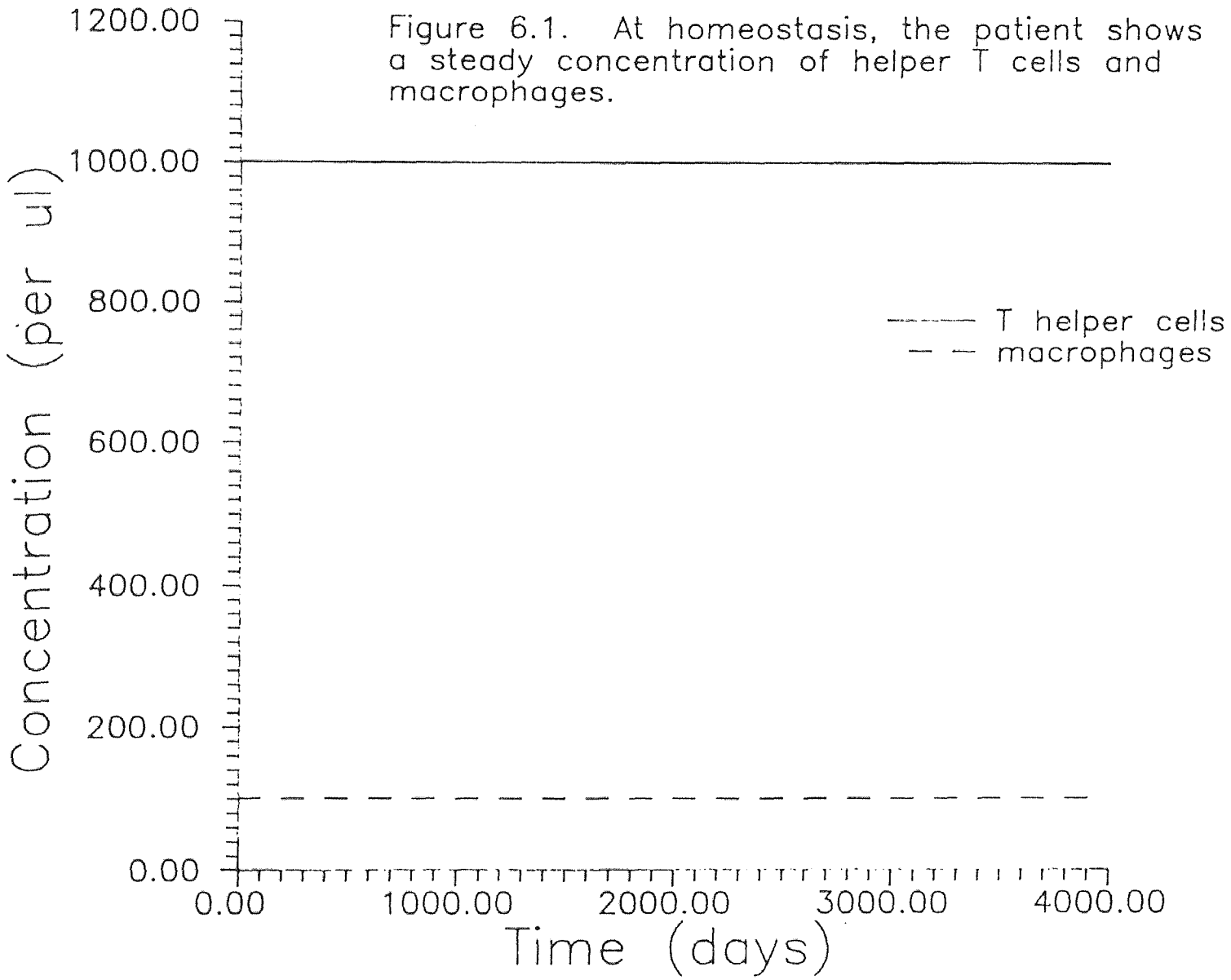


Figure 6.1. At homeostasis, the patient shows a steady concentration of helper T cells and macrophages.

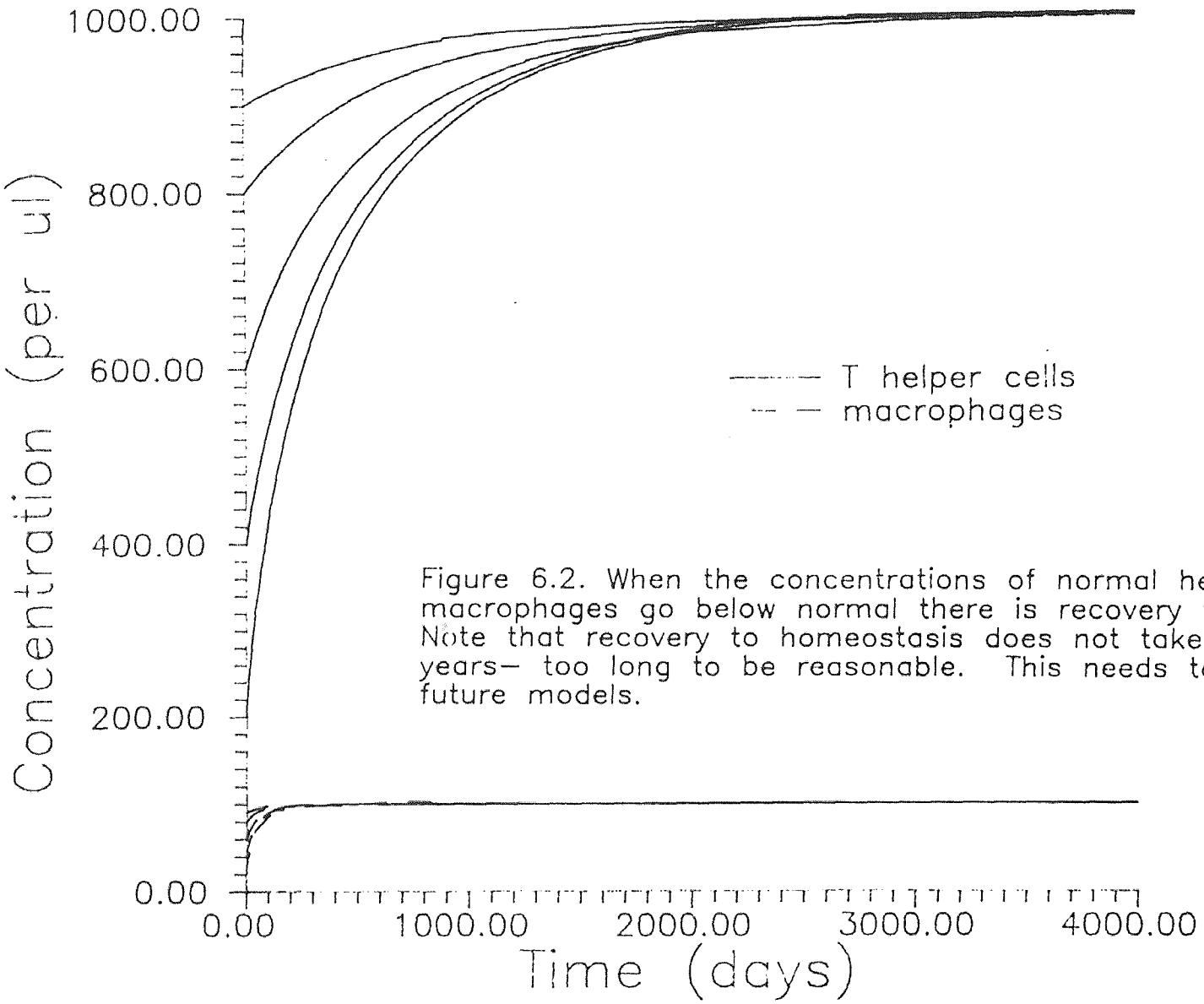


Figure 6.2. When the concentrations of normal helper T cells and macrophages go below normal there is recovery to homeostasis. Note that recovery to homeostasis does not take place for five years— too long to be reasonable. This needs to be modified in future models.

CHAPTER VII

EXPERIMENTAL BACKGROUND

The primary objective of the experimental work was to attain optimization of blood cell separation on an existing blood fractionation device.

Experimental methods have been previously described in detail by Mansoor Kapadvanjwala and Anju Nagpal [19,20].

Briefly, our experiments were performed using bovine blood because its gravitational properties, viscosities and hematocrit are similar to those of human blood and it is a lot safer to use.

The blood was anticoagulated with Acid Citrate Dextrose (ACD) and stored at low temperature until use.

The centrifuge speed was set and kept at about 900 rpm. Approximately 20 ml of blood were pumped into the centrifuge for each run. The blood was pumped into and out of the centrifuge at a pump speed of 200 rpm. Blood was spun in the trapezoidal chamber until definite separation was visible (about 1 hour). Seven samples of blood each containing about 3 ml, were extracted starting from the outer radius of the chamber. The first samples contained the heavy red blood cells. As the withdrawal progressed, less viscous and lower hematocrit blood was collected and at the very end plasma was left in the test tube.

Samples were kept on ice until cell counts of each sample were taken using the Coulter Counter Model CC-180.

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

EXPERIMENTAL RESULTS

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

For this study, three separate runs were performed at the same HCT, centrifuge speed, batch duration and pump withdrawal rate. For each run the leukocyte-rich

cuts were set aside. After three runs, the cuts were combined, the HCT was determined and another separation was performed. This second stage separation was done to obtain better purity of WBC concentration.

Figure 7.1 shows the average results of six first run separations performed on the same day, which yielded two second stage runs.

Figure 7.2 shows a second stage separation performed at a HCT of 40.4. Because the hematocrit is high a high concentration of RBC is still present in the first cut. In Figure 7.3 the results are similar. At a HCT of 32.0 there is still an extremely high concentration of RBC.

The HCT in figure 7.4 is 21.6 and we can see an inversion of the WBC and RBC curves. There is a point of intersection between the curves at this HCT.

When blood was diluted with plasma to lower the HCT to 2.11, there was a total inversion of the curves Figure 7.5. The WBC are now the heavier components because at very low HCT, RBC concentration is low in the suspended plasma medium. Also, WBC are bigger than RBC hence they tend to settle faster.

In conclusion, this extracorporeal detoxification unit could be instrumental in the treatment of CTCL patients by photopheresis. First stage separations of patient blood can be performed. RBC rich blood can be returned to the patient unharmed. Leukocyte rich blood collected from the first stage will then provide an input for the second stage which gives further purity of WBC concentration. Then these purified WBC can be exposed to UVA light in order to destroy the malignant T cells.

If we continued the multistage process of separation it is possible to obtain separation of the various kinds of WBC. This could be instrumental in treatments for CTCL as well as other WBC diseases.

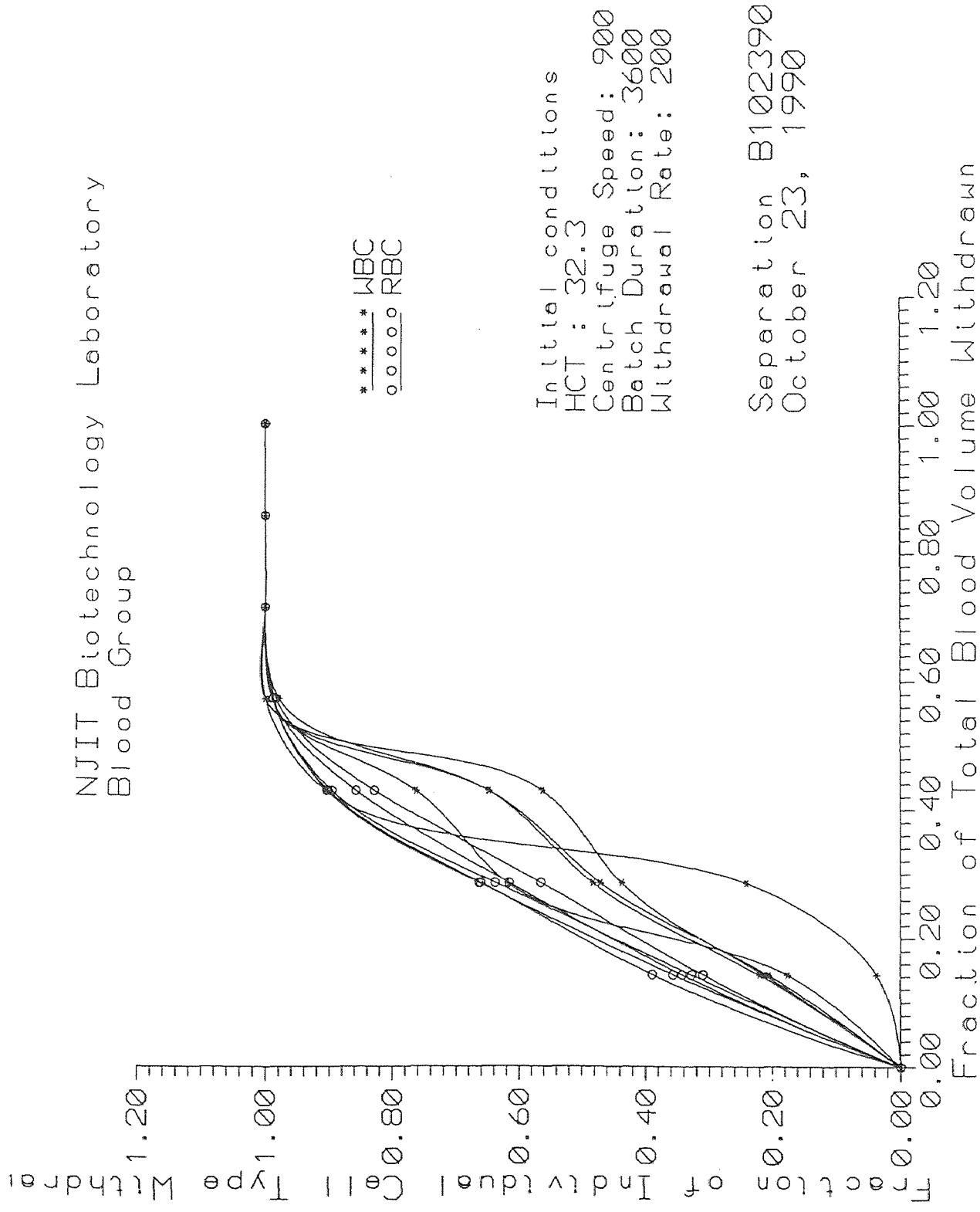

NJIT Biotechnology Laboratory
Blood Group
***** WBC
ooooo RBC
Initial conditions
HCT : 32.3
Centrifuge Speed: 900
Batch Duration: 3600
Withdrawal Rate: 200
Separation B102390
October 23, 1990
Fraction of Total Blood Volume Withdrawn
Fraction of Individual Cell Type Withdrawn
0.00
0.20
0.40
0.60
0.80
1.00
1.20

Figure 7.1

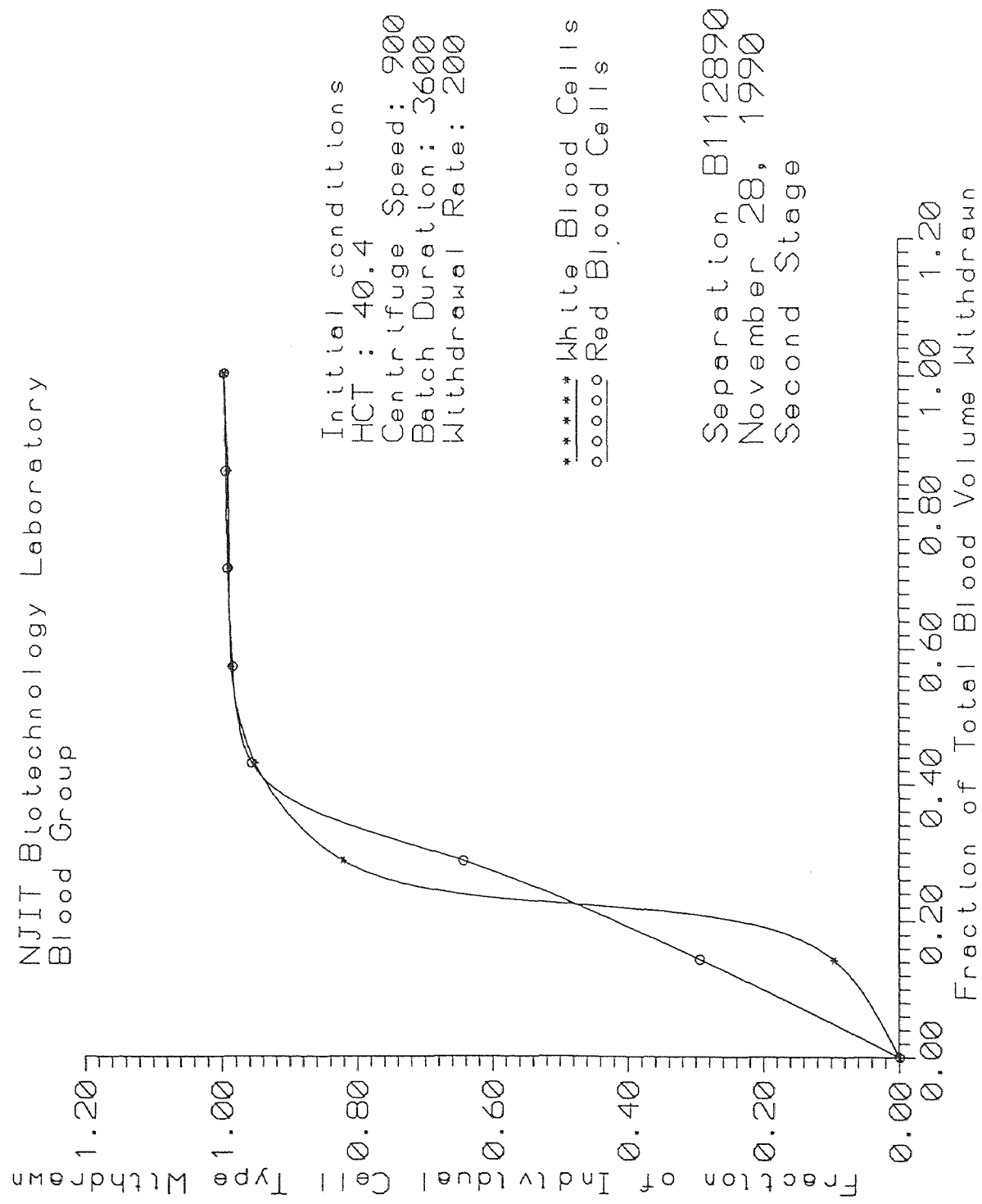

NJIT Biotechnology Laboratory
Blood Group
Initial conditions
HCT : 40.4
Centrifuge Speed: 900
Batch Duration: 3600
Withdrawal Rate: 200
***** White Blood Cells
ooooo Red Blood Cells
Separation B112890
November 28, 1990
Second Stage
Fraction of Individual Cell Type Withdrawn
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Fraction of Total Blood Volume Withdrawn

Figure 7.2

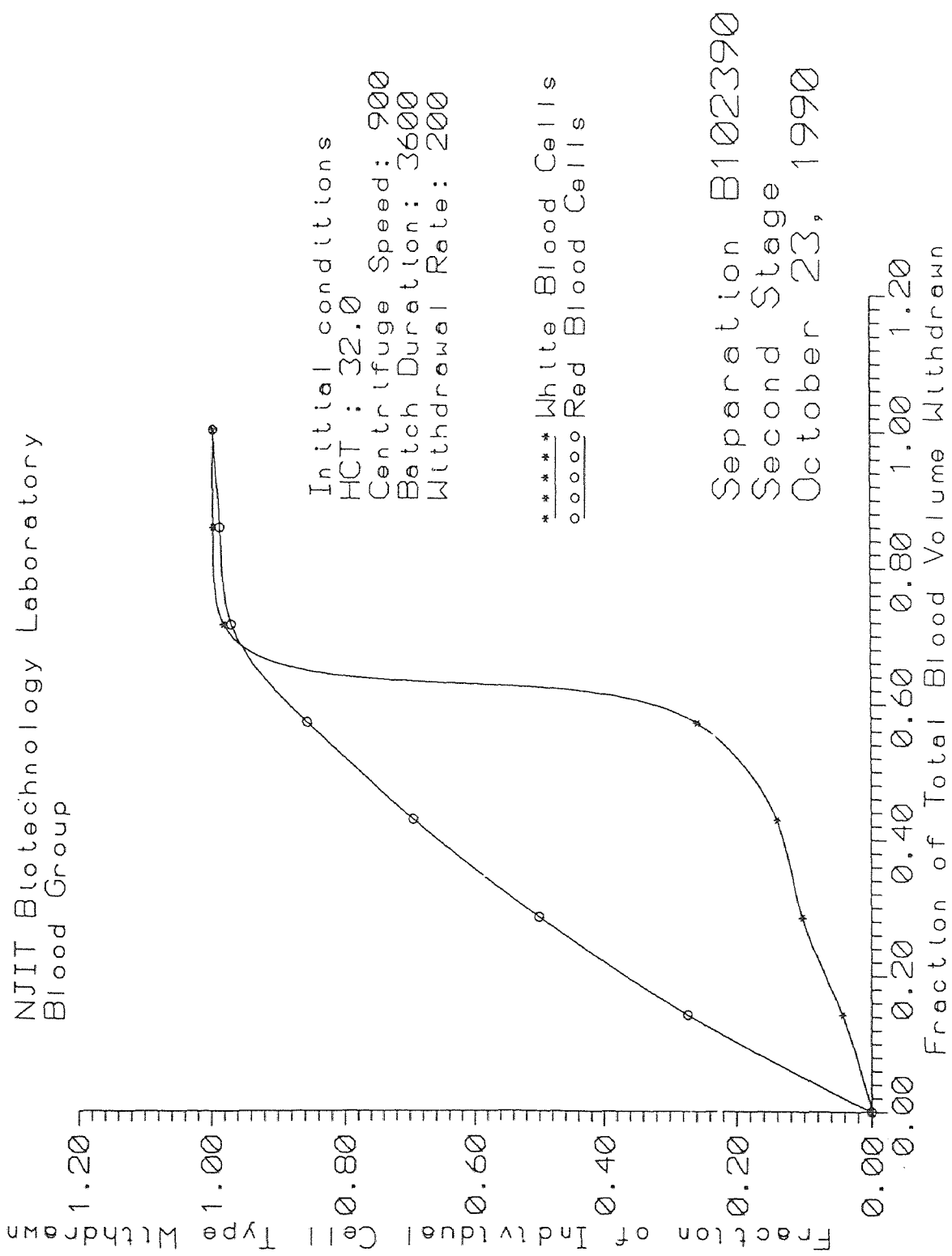

NJIT Biotechnology Laboratory
Blood Group
Initial conditions
HCT : 32.0
Centrifuge Speed: 900
Batch Duration: 3600
Withdrawal Rate: 200
***** White Blood Cells
ooooo Red Blood Cells
Separation B102390
Second Stage
October 23, 1990
Fraction of Total Blood Volume Withdrawn
Fraction of Individual Cell Type Withdrawn
0.00
0.20
0.40
0.60
0.80
1.00
1.20

Figure 7.3

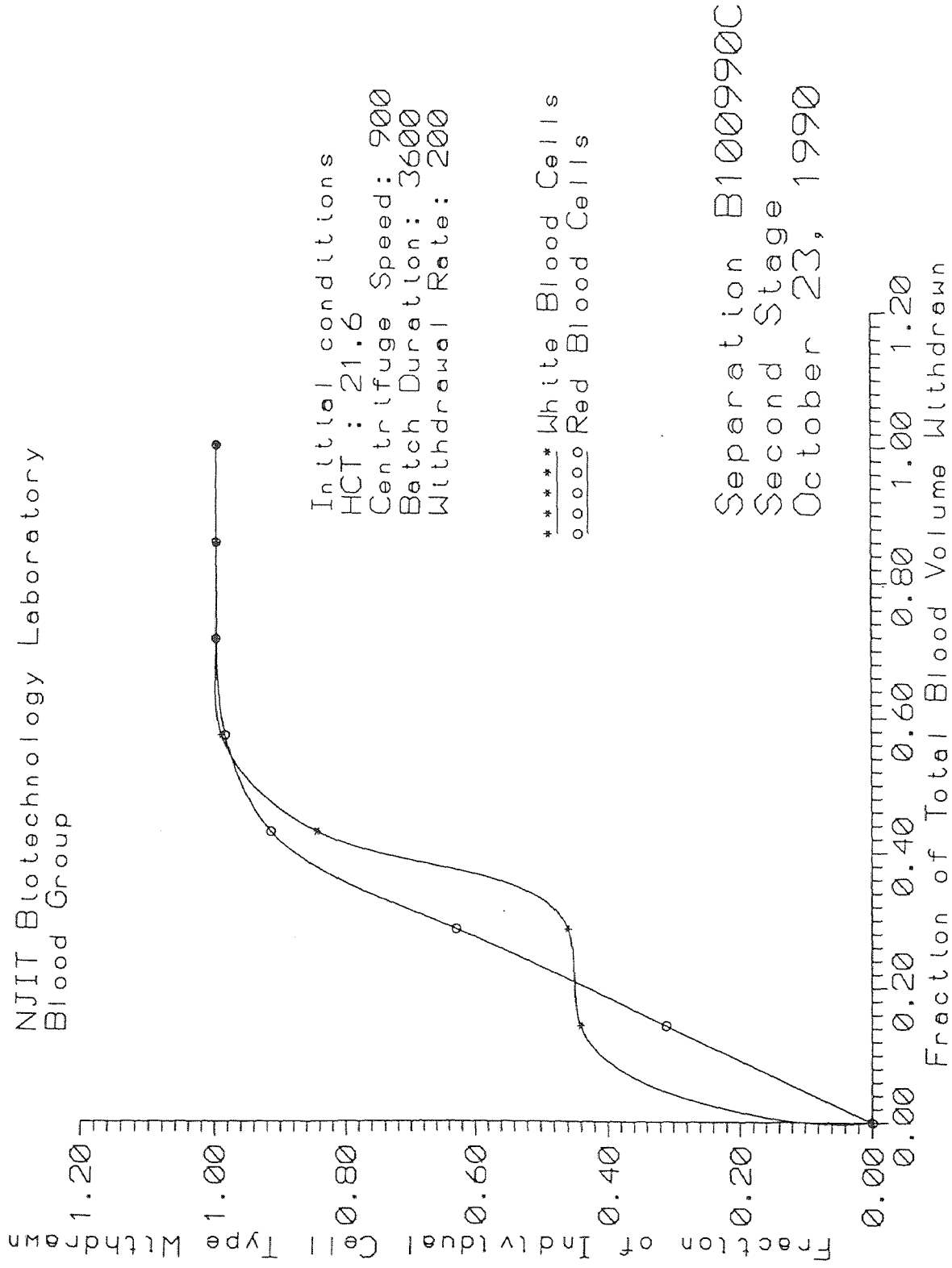

NJIT Biotechnology Laboratory
Blood Group
Initial conditions
HCT : 21.6
Centrifuge Speed: 900
Batch Duration: 3600
Withdrawal Rate: 200
***** White Blood Cells
ooooo Red Blood Cells
Separation B100990C
Second Stage
October 23, 1990
Fraction of Total Blood Volume Withdrawn
Fraction of Individual Cell Type Withdrawn
0.00
0.20
0.40
0.60
0.80
1.00
1.20

Figure 7.4

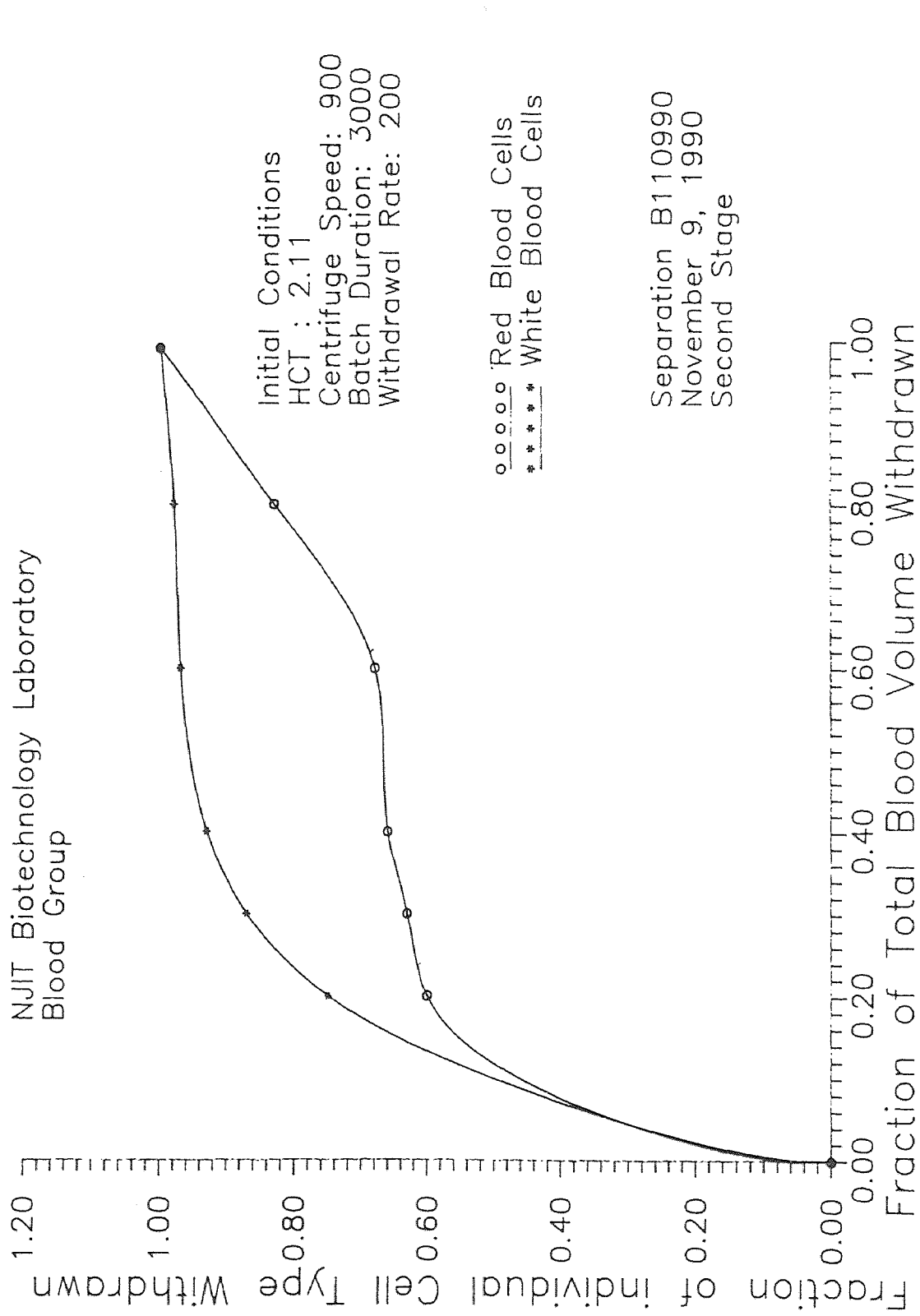

NJIT Biotechnology Laboratory
Blood Group
Initial Conditions
HCT : 2.11
Centrifuge Speed: 900
Batch Duration: 3000
Withdrawal Rate: 200
ooooo Red Blood Cells
***** White Blood Cells
Separation B110990
November 9, 1990
Second Stage
Fraction of Total Blood Volume Withdrawn
Fraction of individual Cell Type Withdrawn
0.00
0.20
0.40
0.60
0.80
1.00
1.20

Figure 7.5

CHAPTER VIII

SIMULATION RESULTS AND DISCUSSION

This thesis provides a mathematical model of the dynamics of CTCL. The model consists of a set of differential equations that have been integrated using a FORTRAN program. The model consists of several parameters which describe the immune changes accompanying the progression of CTCL.

Figure 8.1 shows Base Case results for the parameters obtained in Chapter 6. The disease progresses from homeostasis by injection of carcinogen. It is evident that malignancy begins shortly after carcinogen is introduced into the system. The carcinogen becomes integrated into the DNA of the cell and cancer commences. In Figure 8.1 the concentration of carcinogen begins at 1 and slowly goes to zero. For a healthy person there the cytotoxic T cell concentration is zero but there is a certain concentration of NK cells present-750/ul in our case. In case of malignancy the number of cytotoxic cells and natural killer cells increases in order to engulf the toxic antigen and rid the body of unfavorable substances. Their number, however becomes insignificant when compared to the number of malignant cells. The antibodies increase as malignant T cells increase due to the fact that malignant T cells hyperstimulate B cells to produce antibodies yet their concentration is apparently not enough to cure the patient.

Figure 8.2 shows the change in survival time as the carcinogen strength increases. The greater the concentration of carcinogen in an individuals environment, the faster the death will occur. We have arbitrarily defined death as 10,000 malignant cells per ul of blood.

Figures 8.3-8.10 are a parametric study of the model. The production rates of T cytotoxic cells, antibodies and macrophages were varied to study their effects on the system. Figures 8.3-8.6 differ in the production rate of antibodies.

The study performed on antibodies show that if antibodies concentration goes beyond a certain point (about 4000/ul) there is cure. We called that the critical antibody concentration. For the base case, antibody concentration was below 4000/ul and there was death of the patient. This suggests that if CTCL patients were given

high doses of antibodies specific to the disease, their chances of complete remission could increase.

In Figures 8.7-8.10 show us what happens as the production rate of cytotoxic T and NK cells is increased. For the base case Figure 8.1, the cytotoxic T and NK cell concentration increased only slightly. The effect of the increase is minimal because there is a tremendous amount of malignant cells that need to be engulfed. As the production increases, the patient's survival time increases as shown in Figure 8.11. For rates beyond a certain point, in this case, for values greater than k5 = 0.0001, there is "cure" as seen in Figures 8.7-8.10. The higher the rates of T and NK cell production, the faster the patient is cured.

Macrophages become ineffective when malignant cells begin to proliferate because neoplastic cells produce macrophage inhibitory factor which interferes with their phagocytic and antigen presenting capabilities.

In the model treatment was incorporated by substituing values other than zero for k20 and k21. Figure 8.12 shows a simulation for photopheresis. We see that the concentration of malignant cells is greatly reduced with treatment and cure is achieved. These results were obtained by maintaining k21 =0.0 and making k20 = 0.001. Photophoresis treatment is only targeted at malignant cells, not normal cells. For that reason, we added a negative rate constant to the equation.

In leukapheresis both normal cells and malignant cells are removed. For that reason we included both k20 and k21 for this simulation. The rate of k20 was not changed from the photopheresis simulation. We first made the rate k21 = k20 so that both malignant and normal cells were being removed at the same rate. For this case Tn did not decrease significantly and Tm peaked at 2277 at 4900 days. As k21 was decreased to 0.01, 0.05 and 0.09 respectively, the number of normal cells decreased dramatically but Tm still peaked a about the same concentration at slightly higher time values. When k21 was made 100 times smaller than k20 normal cells decreased from 1000-76 and malignant cells went negative early on.

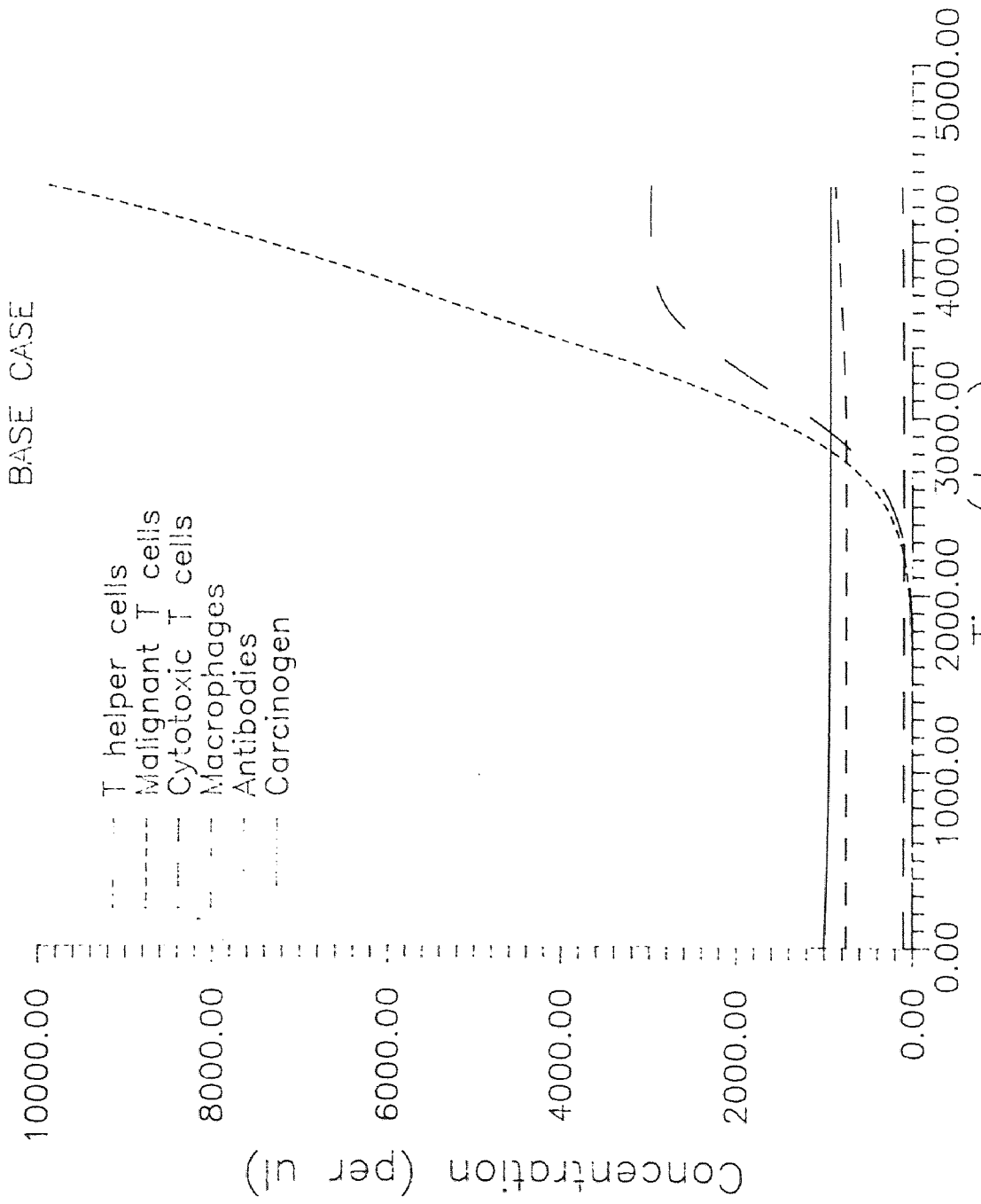

BASE CASE
T helper cells
Malignant T cells
Cytotoxic T cells
Macrophages
Antibodies
Carcinogen
Concentration (per ul)
10000.00
8000.00
6000.00
4000.00
2000.00
0.00
0.00
1000.00
2000.00
3000.00
4000.00
5000.00
Time (days)

Figure 8.1

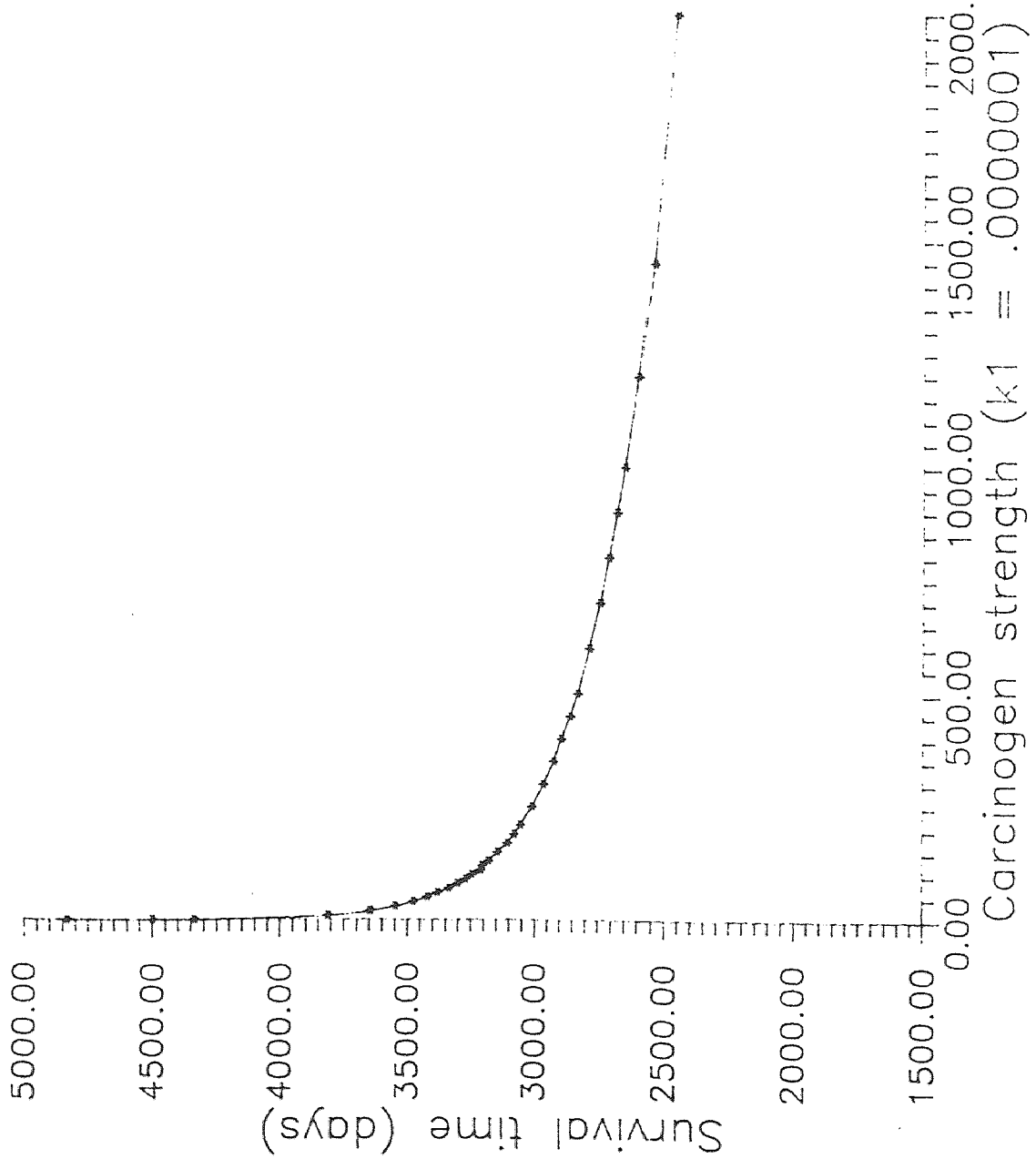

Survival time (days)
5000.00
4500.00
4000.00
3500.00
3000.00
2500.00
2000.00
1500.00
0.00
500.00
1000.00
1500.00
2000.00
Carcinogen strength (k1 = .00000001)

Figure 8.2

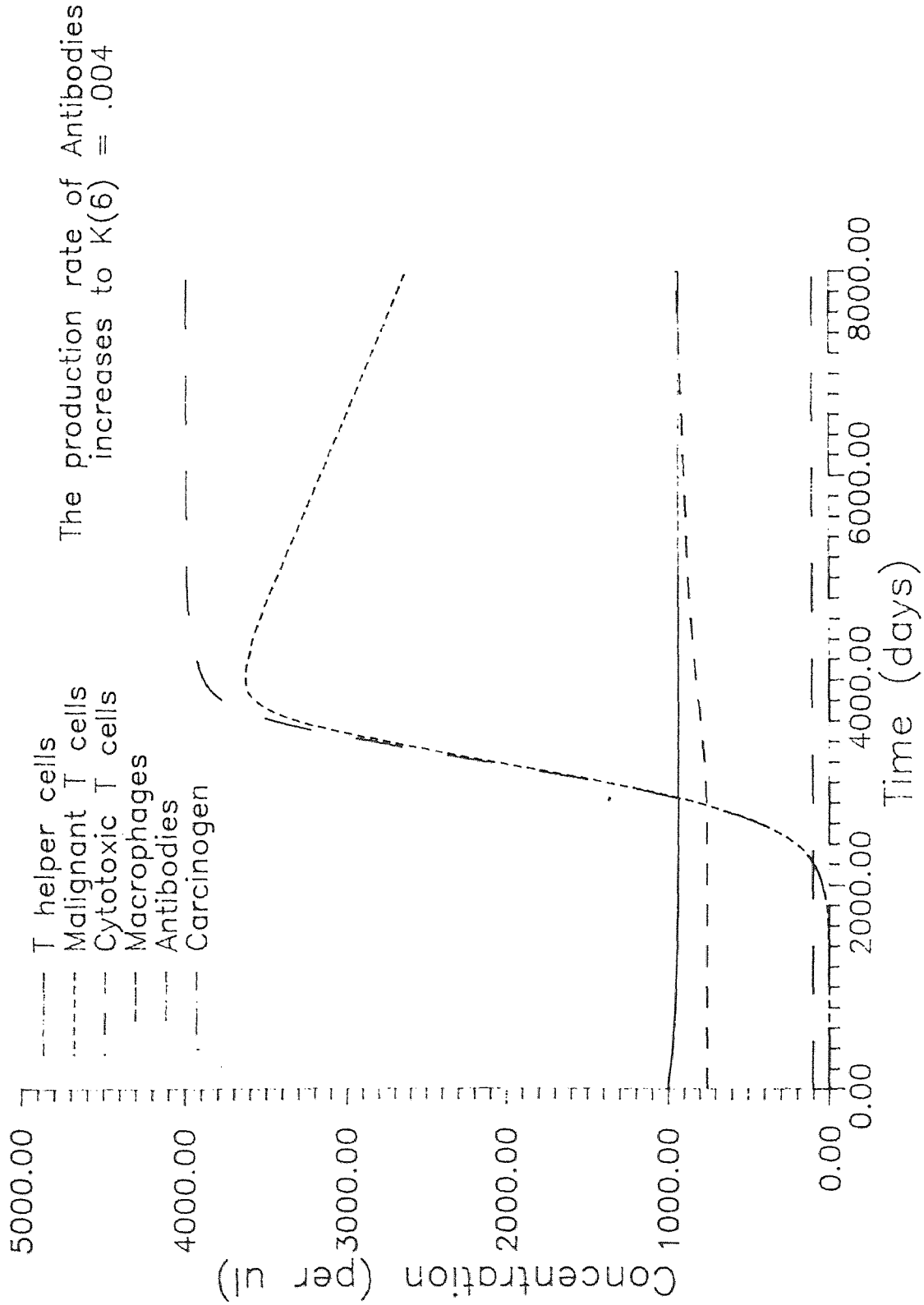

The production rate of Antibodies
increases to K(6) = .004
T helper cells
Malignant T cells
Cytotoxic T cells
Macrophages
Antibodies
Carcinogen
Concentration (per ul)
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
0.00
2000.00
4000.00
6000.00
8000.00
Time (days)

Figure 8.3

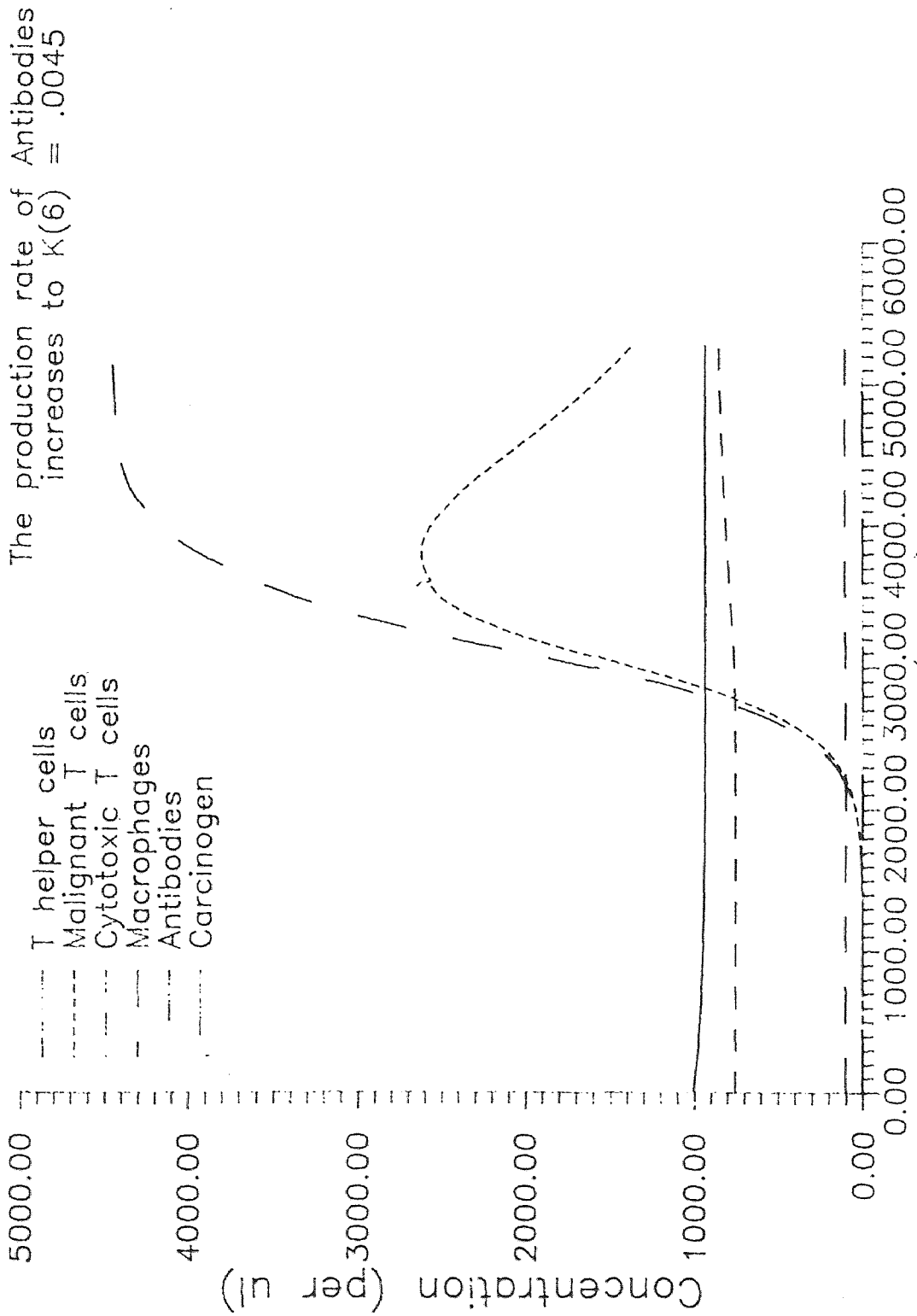

The production rate of Antibodies
increases to K(6) = .0045
T helper cells
Malignant T cells
Cytotoxic T cells
Macrophages
Antibodies
Carcinogen
Concentration (per ul)
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
0.00
1000.00
2000.00
3000.00
4000.00
5000.00
6000.00
Time (days)

Figure 8.4

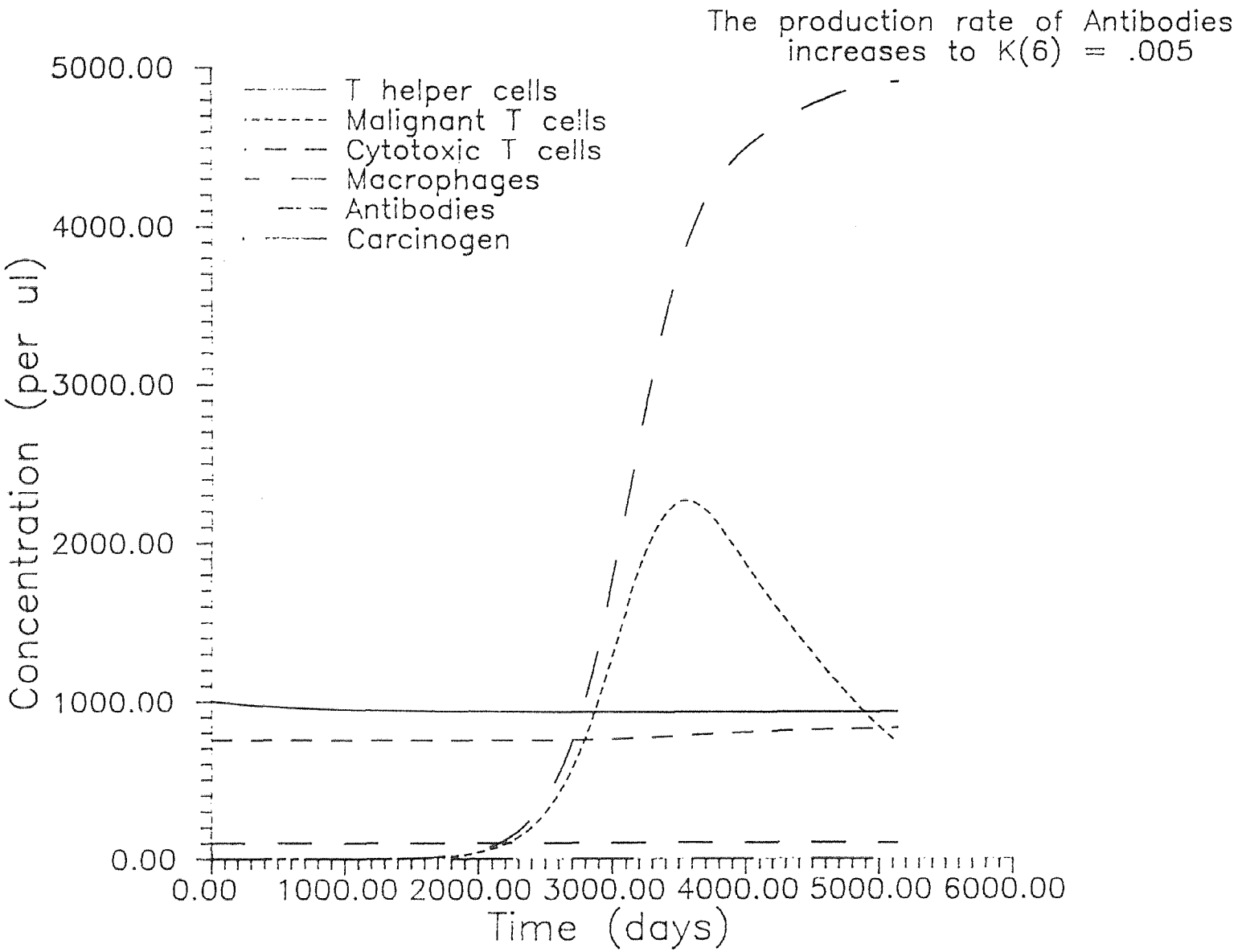

The production rate of Antibodies
increases to K(6) = .005
T helper cells
Malignant T cells
Cytotoxic T cells
Macrophages
Antibodies
Carcinogen
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
Concentration (per ul)
0.00
1000.00
2000.00
3000.00
4000.00
5000.00
6000.00
Time (days)

Figure 8.5

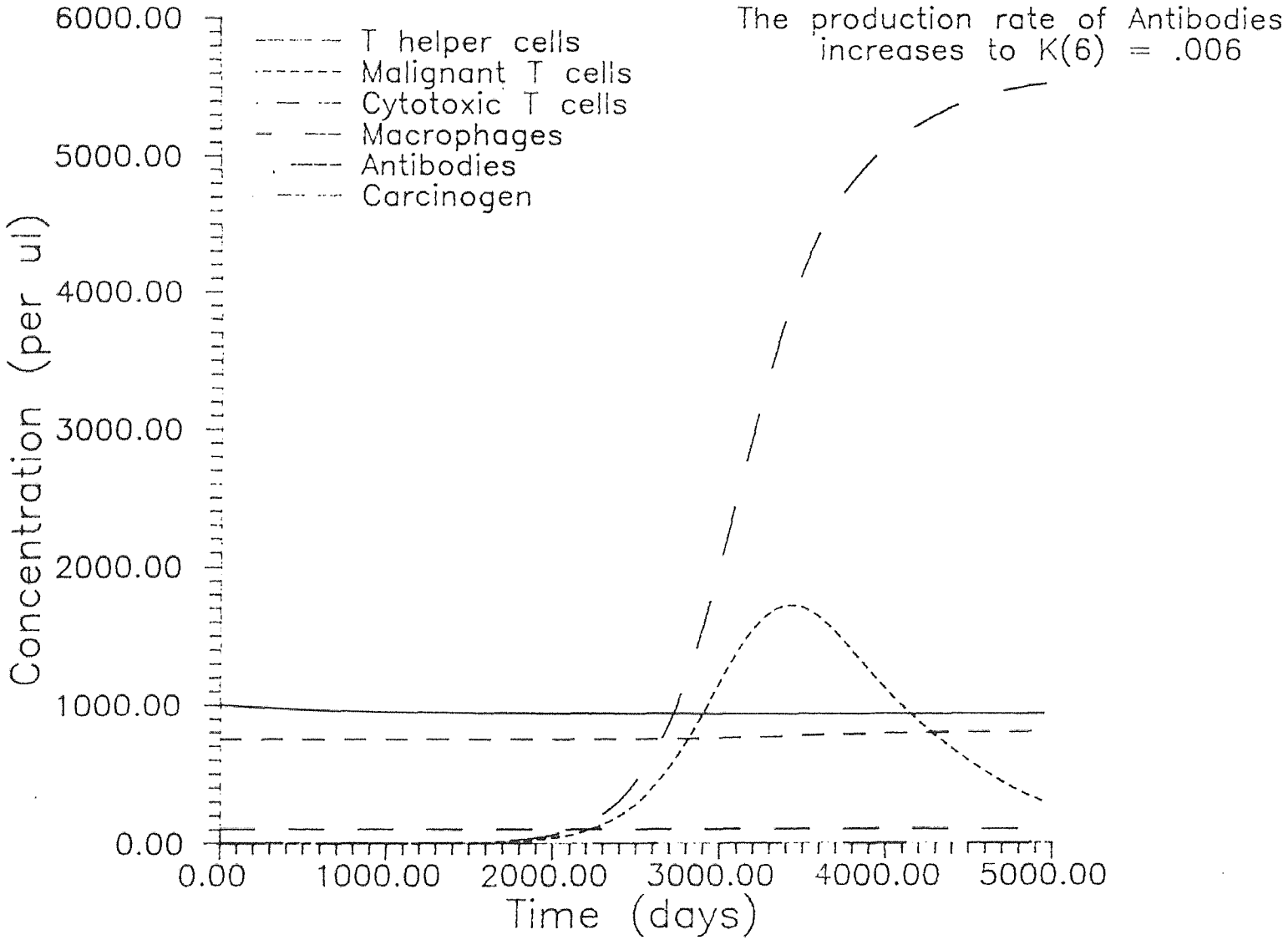

The production rate of Antibodies increases to K(6) = .006
T helper cells
Malignant T cells
Cytotoxic T cells
Macrophages
Antibodies
Carcinogen
Concentration (per ul)
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
0.00
1000.00
2000.00
3000.00
4000.00
5000.00
Time (days)

Figure 8.6

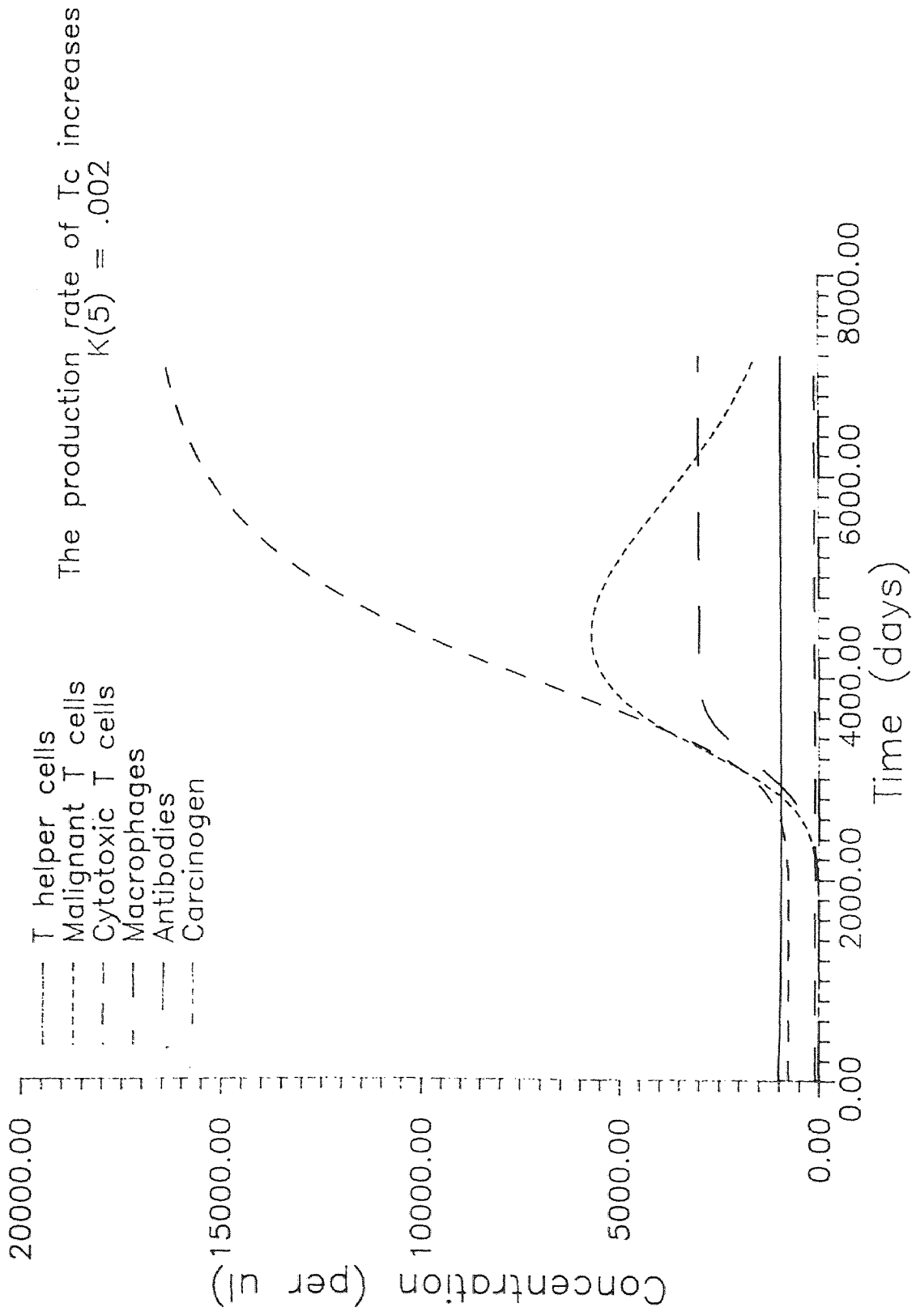

The production rate of Tc increases
K(5) = .002
T helper cells
Malignant T cells
Cytotoxic T cells
Macrophages
Antibodies
Carcinogen
Concentration (per ul)
20000.00
15000.00
10000.00
5000.00
0.00
0.00
2000.00
4000.00
6000.00
8000.00
Time (days)

Figure 8.7

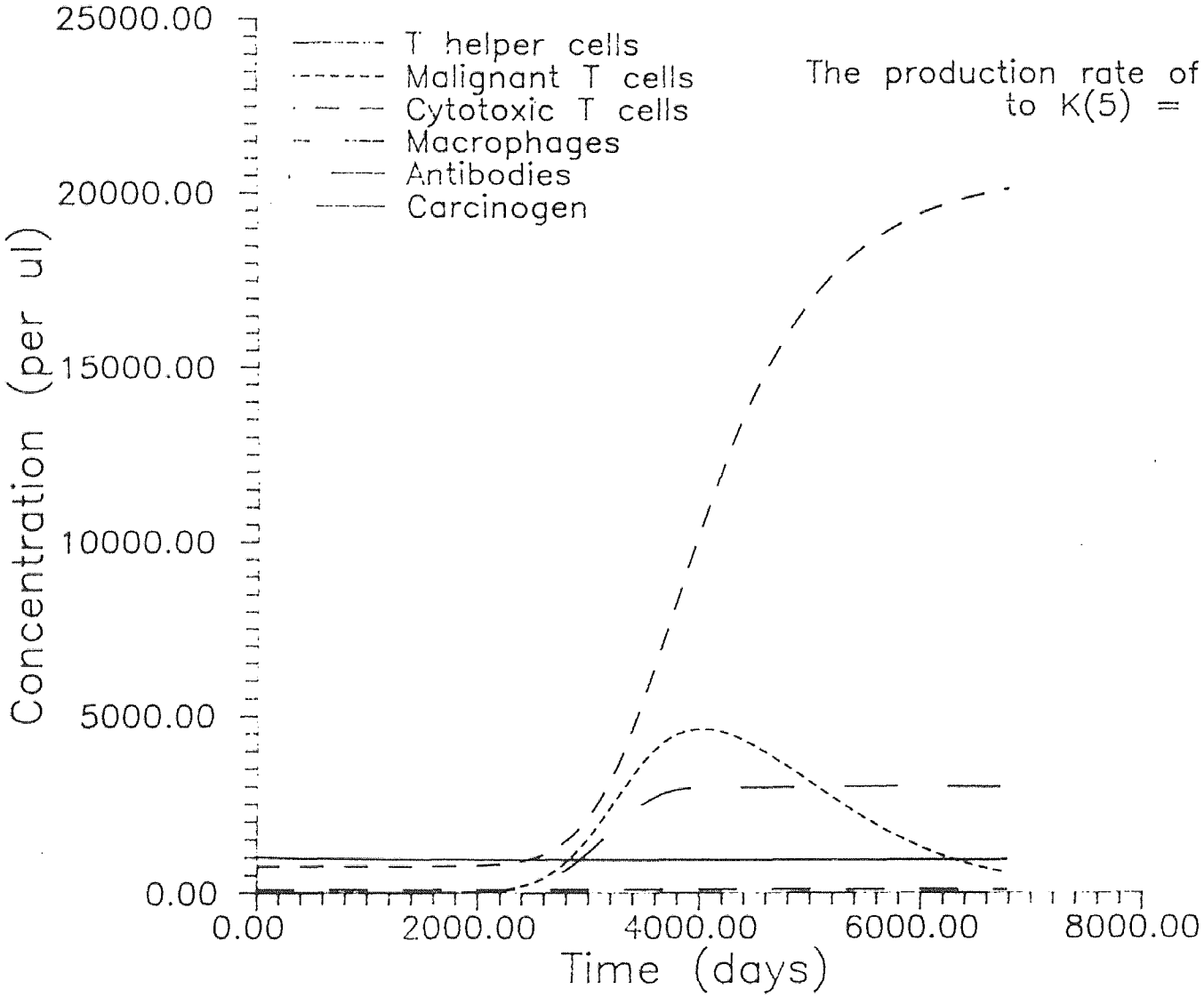

25000.00
20000.00
15000.00
10000.00
5000.00
0.00
Concentration (per ul)
T helper cells
Malignant T cells
Cytotoxic T cells
Macrophages
Antibodies
Carcinogen
The production rate of Tc increases
to K(5) = .003
0.00
2000.00
4000.00
6000.00
8000.00
Time (days)

Figure 8.8

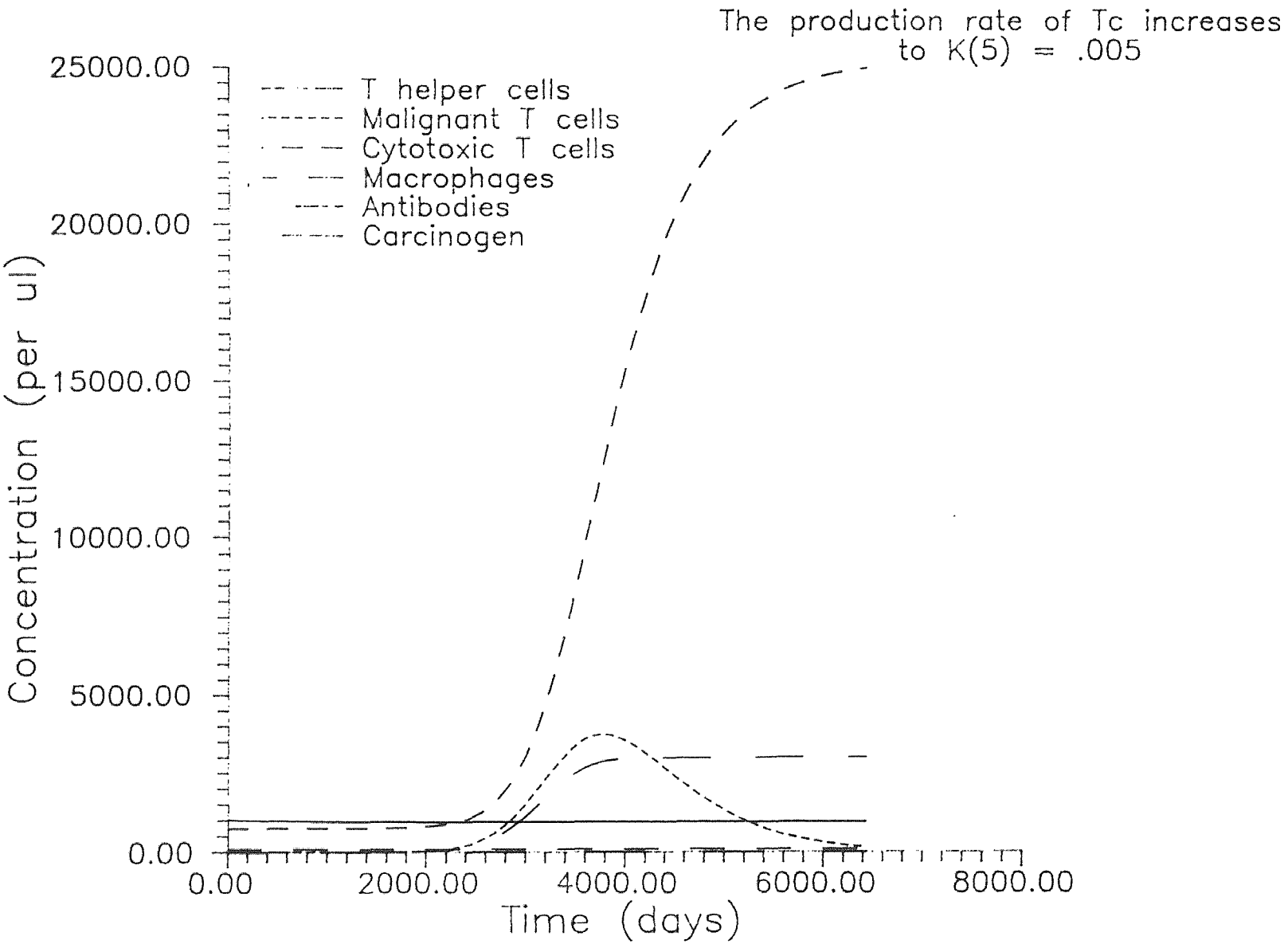

The production rate of Tc increases
to K(5) = .005
T helper cells
Malignant T cells
Cytotoxic T cells
Macrophages
Antibodies
Carcinogen
25000.00
20000.00
15000.00
10000.00
5000.00
0.00
Concentration (per ul)
0.00
2000.00
4000.00
6000.00
8000.00
Time (days)

Figure 8.9

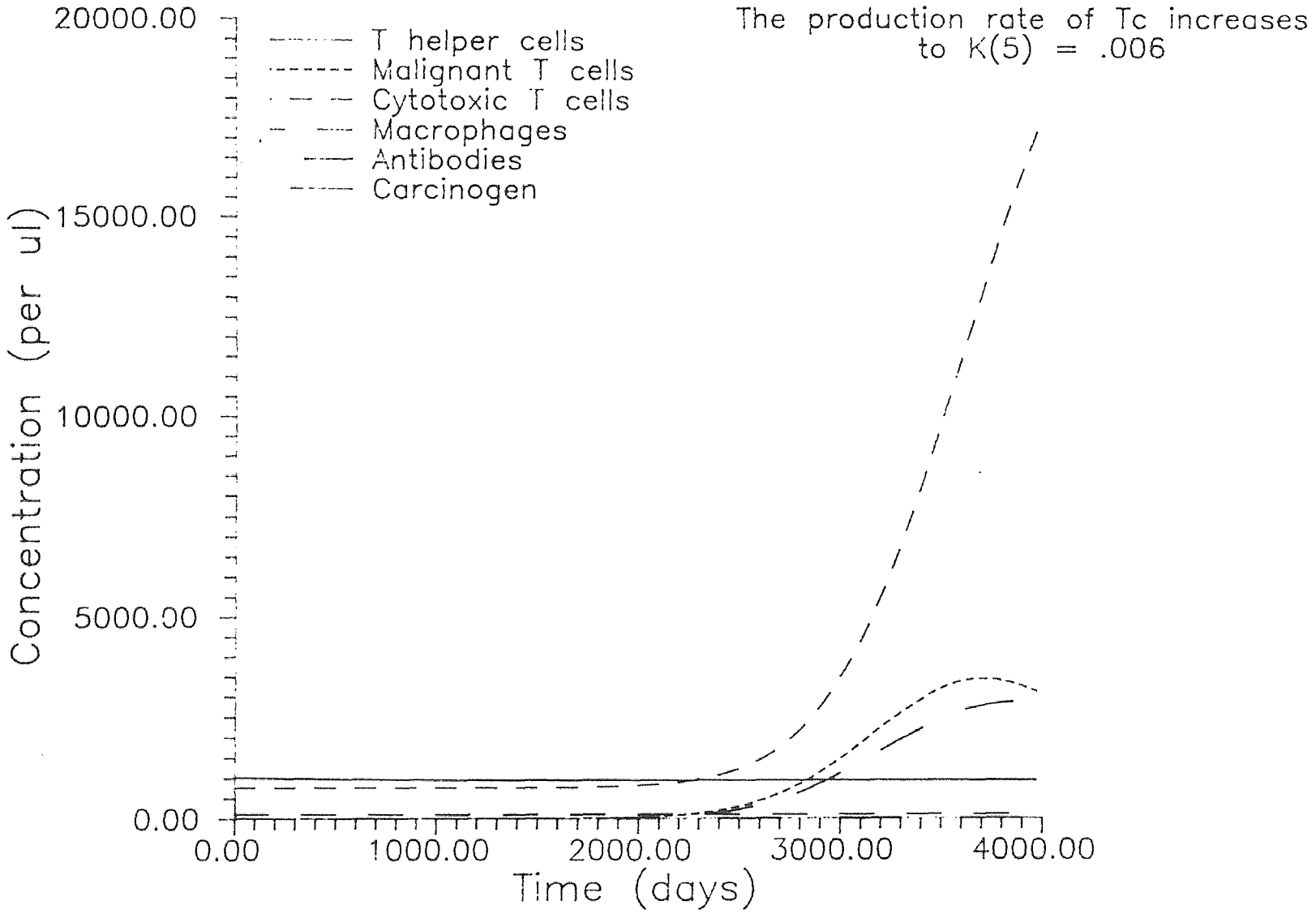

The production rate of Tc increases
to K(5) = .006
T helper cells
Malignant T cells
Cytotoxic T cells
Macrophages
Antibodies
Carcinogen
Concentration (per ul)
20000.00
15000.00
10000.00
5000.00
0.00
0.00
1000.00
2000.00
3000.00
4000.00
Time (days)

Figure 8.10

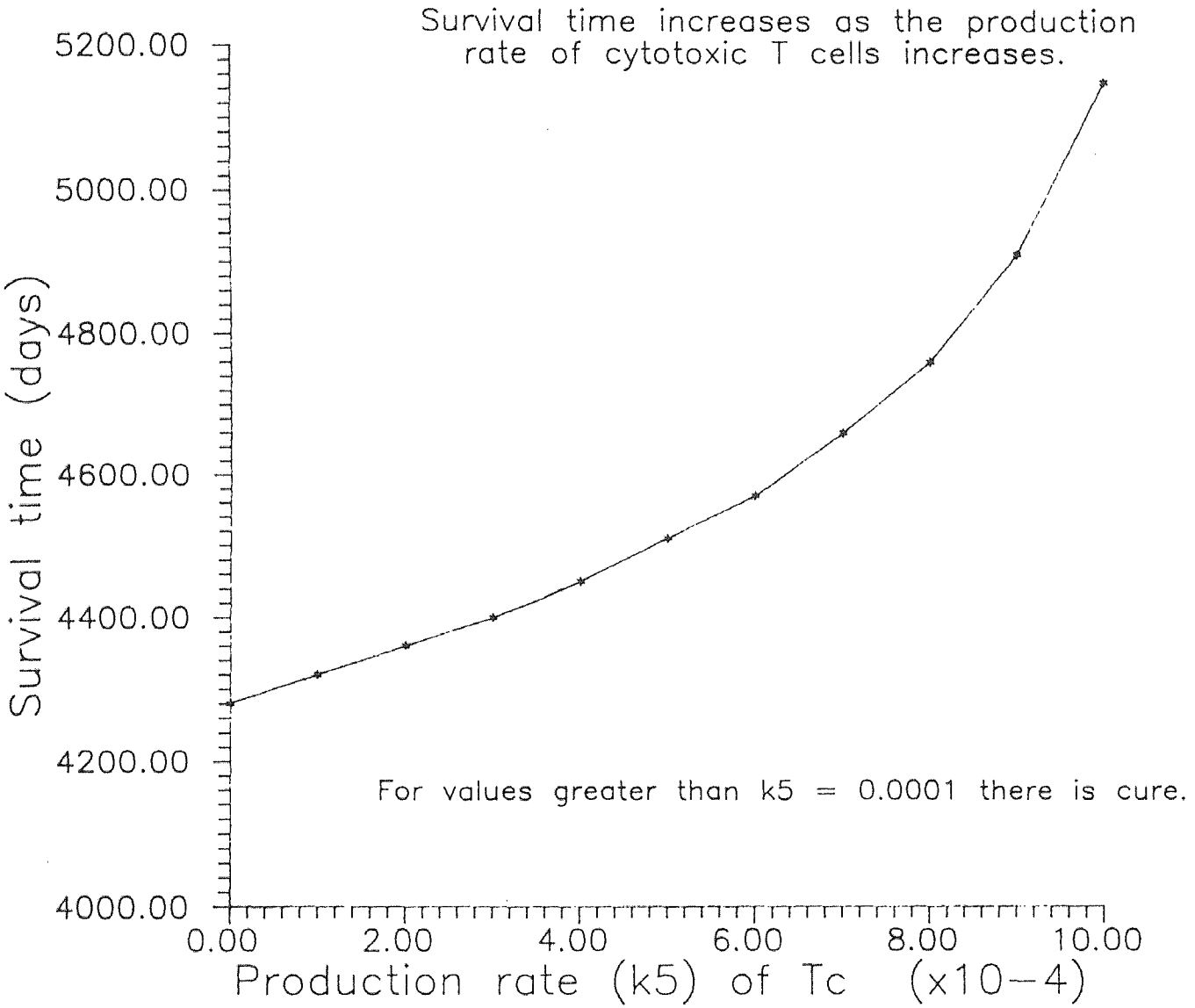

Survival time increases as the production rate of cytotoxic T cells increases.
5200.00
5000.00
4800.00
4600.00
4400.00
4200.00
4000.00
Survival time (days)
For values greater than k5 = 0.0001 there is cure.
0.00
2.00
4.00
6.00
8.00
10.00
Production rate (k5) of Tc (x10-4)

Figure 8.11

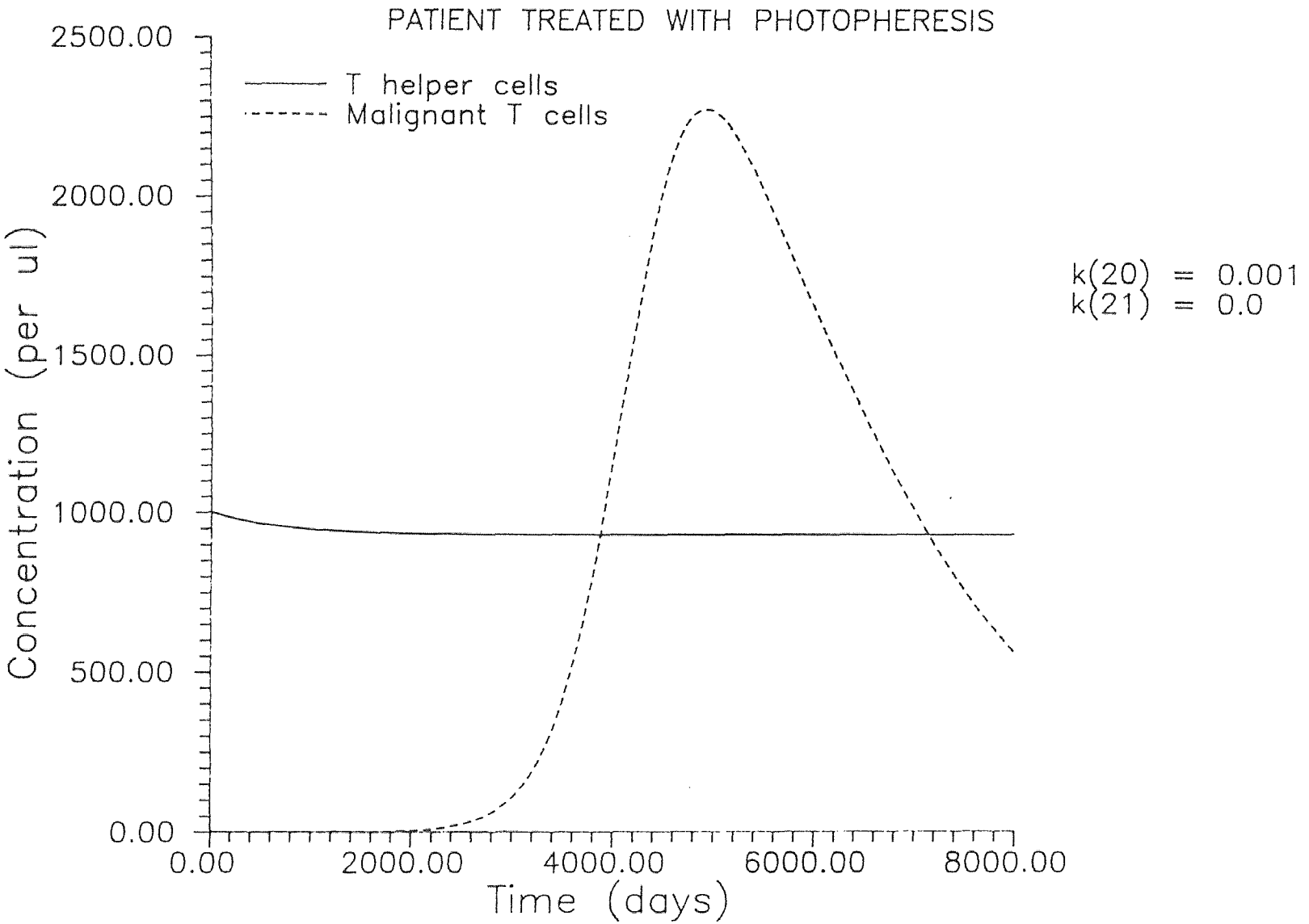

PATIENT TREATED WITH PHOTOPHERESIS
T helper cells
Malignant T cells
k(20) = 0.001
k(21) = 0.0
Concentration (per ul)
2500.00
2000.00
1500.00
1000.00
500.00
0.00
0.00
2000.00
4000.00
6000.00
8000.00
Time (days)

Figure 8.12

CHAPTER IX
CONCLUSIONS

This model simulates the introduction of carcinogen, the transformation of normal cells into malignant cells and subsequent proliferation of malignant cells. The greater the concentration of carcinogen, the faster the transformation between normal cells and malignant cells will occur decreasing survival. Malignant cells multiply so rapidly that immune cells are unable to keep up. They ultimately become diluted and cell-mediated immunity is impaired.

A homeostasis profile has been derived, and responds adequately to perturbation. A Base Case fatal disease simulation has been developed, and sensitivity to carcinogen strength, antibodies, and cytotxic and NK cells has been studied.

The strength of this model lies in its simplicity and flexibility. It can be used in the design of extracorporeal devices which is one thrust of the Blood Group at NJIT Biotechnology Laboratory. The model is also very flexible and could be easily improved by communicating with other colleagues, such as Dr. Emil Bissaccia at Morristown Memorial Hospital.

One weakness in this model is that we assumed a single compartment model. The model doesn't account for the blood-brain barrier which is a drawback in pharmakokinetics. Another weakness is that the constants are patient specific and can't be applied to the general public. The constants vary even within the same patient.

Therefore, when evaluations of photopheresis or leukapheresis are made, it is important to keep in mind the arbitariness of such models. Photopheresis and leukapheresis treatment considerations are only intended as examples of how this model can be implemented as a tool - not as predictions of patient fate.

APPENDIX

This appendix provides a listing of the program used for solving the differential equations in this project.

The input listing for Base Case is given. The output vaules have been previously graphed in Figure 8.1.

```
K(1)  = 1.D-7
K(2)  = 0.0D0
K(3)  = 4.D-3
K(4)  = 5.D-5
K(5)  = 1.D-4
K(6)  = 3.D-3
K(7)  = 1.D-10
K(8)  = 1.D-7
K(9)  = 1.D-6
K(10) = 1000.D0
K(11) = 50.D0
K(12) = 1.9048D0
K(13) = 1000.D0
K(14) = 4.D-6
K(15) = 0.1D0
K(16) = 50.D0
K(17) = 4.D0
K(18) = 100.D0
K(19) = 300.D0
K(20) = 0.0D0
K(21) = 0.0D0
Y(1)  = 1000.D0
Y(2)  = 0.0D0
Y(3)  = 750.D0
Y(4)  = 100.D0
Y(5)  = 0.0D0
Y(6)  = 1.D0

BASE CASE VALUES
```

```
C
C COMMUNICATION BETWEEN THE USER AND THE LSODE PACKAGE, FOR NORMAL
C SITUATIONS, IS SUMMARIZED BELOW.  SEE THE FULL DESCRIPTION FOR
C DETAILS, INCLUDING OPTIONAL COMMUNICATION, NONSTANDARD OPTIONS,
C AND INSTRUCTIONS FOR SPECIAL SITUATIONS.  SEE ALSO THE EXAMPLE
C PROBLEM (WITH PROGRAM AND OUTPUT) FOLLOWING THIS SUMMARY.
C
C A. FIRST PROVIDE A SUBROUTINE OF THE FORM..
C               SUBROUTINE F (NEQ, T, Y, YDOT)
C               DIMENSION Y(NEQ), YDOT(NEQ)
C WHICH SUPPLIES THE VECTOR FUNCTION F BY LOADING YDOT(I) WITH F(I).
C
C B. NEXT DETERMINE (OR GUESS) WHETHER OR NOT THE PROBLEM IS STIFF.
C STIFFNESS OCCURS WHEN THE JACOBIAN MATRIX DF/DY HAS AN EIGENVALUE
C WHOSE REAL PART IS NEGATIVE AND LARGE IN MAGNITUDE, COMPARED TO THE
C RECIPROCAL OF THE T SPAN OF INTEREST.  IF THE PROBLEM IS NONSTIFF,
C USE A METHOD FLAG MF = 10.  IF IT IS STIFF, THERE ARE FOUR STANDARD
C CHOICES FOR MF, AND LSODE REQUIRES THE JACOBIAN MATRIX IN SOME FORM.
C THIS MATRIX IS REGARDED EITHER AS FULL (MF = 21 OR 22),
C OR BANDED (MF = 24 OR 25).  IN THE BANDED CASE, LSODE REQUIRES TWO
C HALF-BANDWIDTH PARAMETERS ML AND MU.  THESE ARE, RESPECTIVELY, THE
C WIDTHS OF THE LOWER AND UPPER PARTS OF THE BAND, EXCLUDING THE MAIN
C DIAGONAL.  THUS THE BAND CONSISTS OF THE LOCATIONS (I,J) WITH
C I-ML .LE. J .LE. I+MU, AND THE FULL BANDWIDTH IS ML+MU+1.
C
C C. IF THE PROBLEM IS STIFF, YOU ARE ENCOURAGED TO SUPPLY THE JACOBIAN
C DIRECTLY (MF = 21 OR 24), BUT IF THIS IS NOT FEASIBLE, LSODE WILL
C COMPUTE IT INTERNALLY BY DIFFERENCE QUOTIENTS (MF = 22 OR 25).
C IF YOU ARE SUPPLYING THE JACOBIAN, PROVIDE A SUBROUTINE OF THE FORM..
C               SUBROUTINE JAC (NEQ, T, Y, ML, MU, PD, NROWPD)
C               DIMENSION Y(NEQ), PD(NROWPD,NEQ)
C WHICH SUPPLIES DF/DY BY LOADING PD AS FOLLOWS..
C     FOR A FULL JACOBIAN (MF = 21), LOAD PD(I,J) WITH DF(I)/DY(J),
C THE PARTIAL DERIVATIVE OF F(I) WITH RESPECT TO Y(J).  (IGNORE THE
C ML AND MU ARGUMENTS IN THIS CASE.)
C     FOR A BANDED JACOBIAN (MF = 24), LOAD PD(I-J+MU+1,J) WITH
C DF(I)/DY(J), I.E. LOAD THE DIAGONAL LINES OF DF/DY INTO THE ROWS OF
C PD FROM THE TOP DOWN.
C     IN EITHER CASE, ONLY NONZERO ELEMENTS NEED BE LOADED.
C
C D. WRITE A MAIN PROGRAM WHICH CALLS SUBROUTINE LSODE ONCE FOR
C EACH POINT AT WHICH ANSWERS ARE DESIRED.  THIS SHOULD ALSO PROVIDE
C FOR POSSIBLE USE OF LOGICAL UNIT 3 FOR OUTPUT OF ERROR MESSAGES
C BY LSODE.  ON THE FIRST CALL TO LSODE, SUPPLY ARGUMENTS AS FOLLOWS..
C F      = NAME OF SUBROUTINE FOR RIGHT-HAND SIDE VECTOR F.
C          THIS NAME MUST BE DECLARED EXTERNAL IN CALLING PROGRAM.
C NEQ    = NUMBER OF FIRST ORDER ODE-S.
C Y      = ARRAY OF INITIAL VALUES, OF LENGTH NEQ.
C T      = THE INITIAL VALUE OF THE INDEPENDENT VARIABLE.
C TOUT   = FIRST POINT WHERE OUTPUT IS DESIRED (.NE. T).
C ITOL   = 1 OR 2 ACCORDING AS ATOL (BELOW) IS A SCALAR OR ARRAY.
C RTOL   = RELATIVE TOLERANCE PARAMETER (SCALAR).
C ATOL   = ABSOLUTE TOLERANCE PARAMETER (SCALAR OR ARRAY).
C          THE LOCAL ERROR IN Y(I) WILL BE CONTROLLED SO AS TO BE
C          ROUGHLY LESS THAN  RTOL*ABS(Y(I)) + ATOL  IF ITOL = 1,
C          OR  RTOL*ABS(Y(I)) + ATOL(I)  IF ITOL = 2.  USE RTOL = 0.0
C          FOR PURE ABSOLUTE ERROR CONTROL, ATOL (OR ATOL(I)) = 0.0
C          FOR PURE RELATIVE.  CAUTION.. ACTUAL (GLOBAL) ERRORS
C          MAY EXCEED THESE TOLERANCES, SO CHOOSE THEM CONSERVATIVELY.
C ITASK  = 1 FOR NORMAL COMPUTATION OF OUTPUT VALUES OF Y AT T = TOUT.
C ISTATE = INTEGER FLAG (INPUT AND OUTPUT).  SET ISTATE = 1.
```

```
C IOPT   = 0 TO INDICATE NO OPTIONAL INPUTS USED.
C RWORK  = REAL WORK ARRAY OF LENGTH AT LEAST..
C             20 + 16*NEQ                    FOR MF = 10,
C             22 +  9*NEQ + NEQ**2           FOR MF = 21 OR 22,
C             22 + 10*NEQ + (2*ML + MU)*NEQ  FOR MF = 24 OR 25.
C LRW    = DECLARED LENGTH OF RWORK (IN USER-S DIMENSION).
C IWORK  = INTEGER WORK ARRAY OF LENGTH AT LEAST..
C             20         FOR MF = 10,
C             20 + NEQ   FOR MF = 21, 22, 24, OR 25.
C           IF MF = 24 OR 25, INPUT IN IWORK(1),IWORK(2) THE LOWER
C           AND UPPER HALF-BANDWIDTHS ML,MU.
C LIW    = DECLARED LENGTH OF IWORK (IN USER-S DIMENSION).
C JAC    = NAME OF SUBROUTINE FOR JACOBIAN MATRIX (MF = 21 OR 24).
C           IF USED, THIS NAME MUST BE DECLARED EXTERNAL IN CALLING
C           PROGRAM.  IF NOT USED, PASS A DUMMY NAME.
C MF     = METHOD FLAG.  STANDARD VALUES ARE..
C           10 FOR NONSTIFF (ADAMS) METHOD, NO JACOBIAN USED.
C           21 FOR STIFF (BDF) METHOD, USER-SUPPLIED FULL JACOBIAN.
C           22 FOR STIFF METHOD, INTERNALLY GENERATED FULL JACOBIAN.
C           24 FOR STIFF METHOD, USER-SUPPLIED BANDED JACOBIAN.
C           25 FOR STIFF METHOD, INTERNALLY GENERATED BANDED JACOBIAN.
C
C E. THE OUTPUT FROM THE FIRST CALL (OR ANY CALL) IS..
C      Y = ARRAY OF COMPUTED VALUES OF Y(T) VECTOR.
C      T = CORRESPONDING VALUE OF INDEPENDENT VARIABLE (NORMALLY TOUT).
C ISTATE = 2  IF LSODE WAS SUCCESSFUL, NEGATIVE OTHERWISE.
C          -1 MEANS EXCESS WORK DONE ON THIS CALL (PERHAPS WRONG MF).
C          -2 MEANS EXCESS ACCURACY REQUESTED (TOLERANCES TOO SMALL).
C          -3 MEANS ILLEGAL INPUT DETECTED (SEE PRINTED MESSAGE).
C          -4 MEANS REPEATED ERROR TEST FAILURES (CHECK ALL INPUTS).
C          -5 MEANS REPEATED CONVERGENCE FAILURES (PERHAPS BAD JACOBIAN
C             SUPPLIED OR WRONG CHOICE OF MF OR TOLERANCES).
C          -6 MEANS ERROR WEIGHT BECAME ZERO DURING PROBLEM. (SOLUTION
C             COMPONENT I VANISHED, AND ATOL OR ATOL(I) = 0.)
C
C F. TO CONTINUE THE INTEGRATION AFTER A SUCCESSFUL RETURN, SIMPLY
C RESET TOUT AND CALL LSODE AGAIN.  NO OTHER PARAMETERS NEED BE RESET.
C
C-----------------------------------------------------------------------
C
      program IMMUNITY
      parameter(luw=6, lur=5)
      EXTERNAL FEX, JEX
      DOUBLE PRECISION ATOL, RWORK, RTOL, T, TOUT,Y
      DIMENSION Y(6), ATOL(10), RWORK(212), IWORK(30)
      character filename*12
      real*8 K(21)
      common /IMMUNE1_K/ K

c                output to filename which is provide on the command line
c      write(*,*) 'enter datafile name = '
c      read(*,'(a12)') filename
c      filename(9:12)='.in '
c      open(lur,file=filename)
c      filename(9:12)='.out'
c      open(luw,file=filename)
       open(lur,file='immune1.in')
       open(luw,file='immune1.dat')
c                                          initializing the constants
      NEQ = 6
```

```
      do 10 i=1, 21
            read(lur,900) K(i)
10 continue
      write(*,*) 'K = ',(K(i),i=1,21)
      do 20 i=1, 6
            read(lur,900) Y(i)
20 continue
      write(*,*) 'Y = ',(Y(i),i=1,6)
c     K(1) = 234.D0
c     K(2) = 1.D0
c     K(3) = 1.03D0
c     K(4) = 1.D0
c     K(5) = 1600.D0
c     K(6) = 1.D0
c     K(7) = 1.2D0
c     K(8) = 1.D0
c     K(9) = 1.01D0
c     K(10) = 0.2D0
c                              initial conditions for the system variables
c     Y(1) = 300.d0
c     Y(2) = 0.D0
c     Y(3) = 800.D0
c     Y(4) = 10.D0
c     Y(5) = 800.D0
c     Y(6) = 0.D0
c     Y(7) = 0.D0
c     Y(8) = 0.D0
c     Y(9) = 1.D-3
c     Y(10) = 0.D0
c                                              initializing other things
      T = 0.D0
      TOUT = 0.D0
      ITOL = 2
      RTOL = 0.01d0
      ATOL(1) = 1.d-2
      ATOL(2) = 1.d-2
      ATOL(3) = 1.d-2
      ATOL(4) = 1.d-2
      ATOL(5) = 1.d-2
      ATOL(6) = 1.d-2
      ATOL(7) = 1.d-2
      ATOL(8) = 1.d-2
      ATOL(9) = 1.d-2
      ATOL(10) = 1.d-2
      ITASK = 1
      ISTATE = 1
      IOPT = 0
      LRW = 212
      LIW = 30
      MF = 21
c     write(luw,910)

      write(luw,920) T,(Y(i),i=1,6)
c                    call the executive subprogram LSODE and integrate
      DO 40 IOUT = 1,800
        CALL LSODE(FEX,NEQ,Y,T,TOUT,ITOL,RTOL,ATOL,ITASK,ISTATE,
     &             IOPT,RWORK,LRW,IWORK,LIW,JEX,MF)

      WRITE(luw,920)T,(Y(i),i=1,6)
      IF (ISTATE .LT. 0) GO TO 80
```

```
 40     TOUT = TOUT + 10.D0
        WRITE(*,960)IWORK(11),IWORK(12),IWORK(13)
        WRITE(luw,960)IWORK(11),IWORK(12),IWORK(13)
        STOP
 80     WRITE(*,990)ISTATE
        WRITE(luw,990)ISTATE
        STOP
900     format(8x,g30.0)
901     format(1x,'K = ',6(g13.7,' ':))
902     format(1x,'Y = ',5(g13.7,' ':))
910     FORMAT(' Time    Tn         Tm      Tc       M       Ag      C ')
920     FORMAT(f5.0,' ',6(G11.4,' ',))
960     FORMAT(/12H NO. STEPS =,I4,11H  NO. F-S =,I4,11H  NO. J-S =,I4)
990     FORMAT(///22H ERROR HALT.. ISTATE =,I3)
        END



        SUBROUTINE FEX (NEQ, T, Y, YDOT)
        DOUBLE PRECISION T, Y, YDOT
        DIMENSION Y(6), YDOT(6)
        real*8 K(21)
        common /IMMUNE1_K/ K
        IF (Y(2) .GE. 0.D0 .OR. Y(6) .GE. 0.D0) THEN
            YDOT(1) = K(10)/(K(11) + Y(1)) - K(12)*Y(1)/(K(13) + Y(1))
     &              - K(1)*Y(1)*Y(6) - K(15) - K(21)*Y(1)
        ELSE
            YDOT(1) = K(10)/(K(11) + Y(1)) - K(12)*Y(1)/(K(13) + Y(1))
     &              - K(1)*Y(1)*Y(6) - K(21)*Y(1)
        ENDIF
        YDOT(2) = K(1)*Y(1)*Y(6) + K(3)*Y(2) - K(7)*Y(4)*Y(2)
     &           -K(8)*Y(3)*Y(2) - K(9)*Y(5)*Y(2) - K(20)*Y(2)
        YDOT(3) = K(5)*Y(2) - K(8)*Y(3)*Y(2)
        YDOT(4) = K(19)/(K(16) + Y(4)) - K(17)*Y(4)/(K(18) + Y(4))
     &          - K(7)*Y(4)*Y(2) + K(4)*Y(2)
        YDOT(5) = K(6)*Y(2) - K(9)*Y(5)*Y(2)
        YDOT(6) = - K(14)*Y(6)*Y(1)
        RETURN
        END

        SUBROUTINE JEX (NEQ, T, Y, ML, MU, PD, NRPD)
        DOUBLE PRECISION PD, T, Y
        DIMENSION Y(6), PD(NRPD,6)
        real*8 K(21)
        common /IMMUNE1_K/ K
        PD(1,1) = - K(10)/(K(11)+Y(1))**2 - (K(12)*K(13))/(K(13)+Y(1))**2
     &          - K(1)*Y(6) - K(21)
        PD(1,2) = 0.0
        PD(1,3) = 0.0
        PD(1,4) = 0.0
        PD(1,5) = 0.0
        PD(1,6) = - K(1)*Y(1)
        PD(2,1) = K(1)*Y(6)
        PD(2,2) = K(3) - K(7)*Y(4) - K(8)*Y(3) - K(9)*Y(5) - K(20)
        PD(2,3) = - K(8)*Y(2)
        PD(2,4) = - K(7)*Y(2)
        PD(2,5) = - K(9)*Y(2)
        PD(2,6) = K(1)*Y(2)
        PD(3,1) = 0.0
        PD(3,2) = K(5) - K(8)*Y(3)
```

```
      PD(3,3) = - K(8)*Y(2)
      PD(3,4) = 0.0
      PD(3,5) = 0.0
      PD(3,6) = 0.0
      PD(4,1) = 0.0
      PD(4,2) = K(4) - K(7)*Y(4)
      PD(4,3) = 0.0
      PD(4,4) = - K(19)/(K(16)+Y(4))**2 - (K(17)*K(18))/(K(18)+Y(4))**2
     &          - K(7)*Y(2)
      PD(4,5) = 0.0
      PD(4,6) = 0.0
      PD(5,1) = 0.0
      PD(5,2) = K(6) - K(9)*Y(5)
      PD(5,3) = 0.0
      PD(5,4) = 0.0
      PD(5,5) = - K(9)*Y(2)
      PD(5,6) = 0.0
      PD(6,1) = - K(14)*Y(6)
      PD(6,2) = 0.0
      PD(6,3) = 0.0
      PD(6,4) = 0.0
      PD(6,5) = 0.0
      PD(6,6) = - K(14)*Y(1)
      RETURN
      END
```

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