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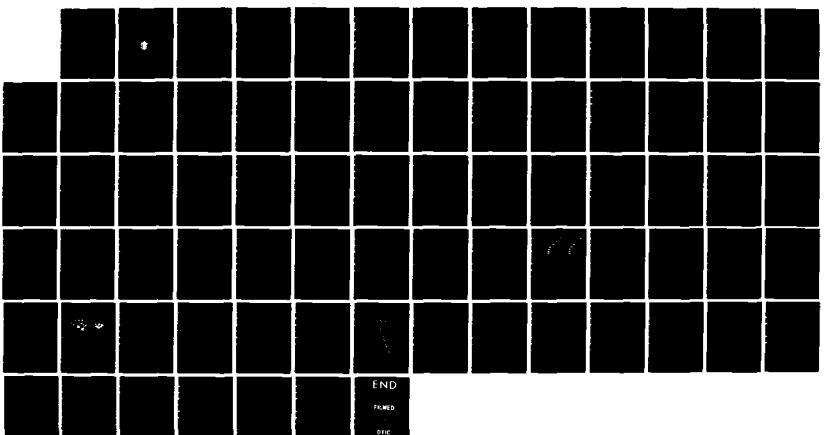

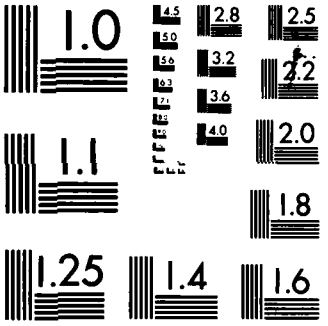

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A generalized kinetic model has been developed which describes the T-cell independent antibody-mediated primary immune response. Immunology is a very young science and its history is important to understand the direction of investigation of those questions which remain unsolved. The immune system itself, with all the cells, cellular products, and lymph system, is very complex, second only to the nervous system for complexity in the human body.

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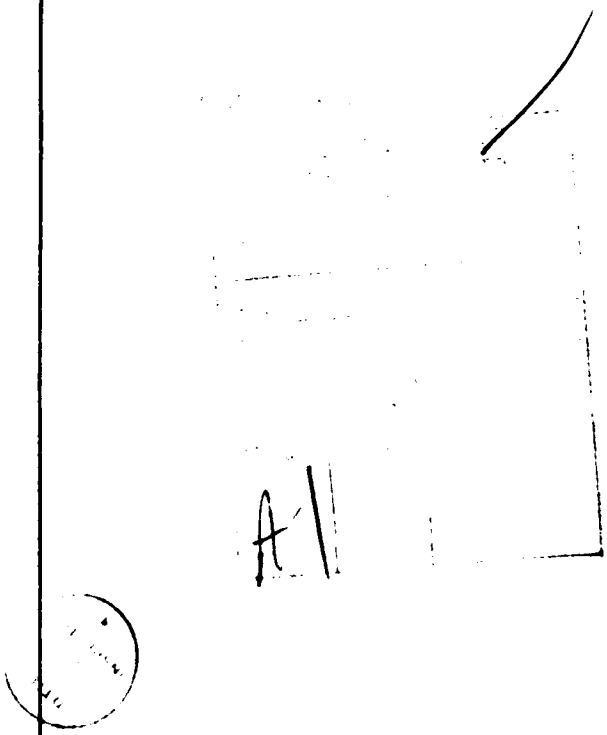
The original theoretical kinetic model was developed by Bell in 1970. However there have been many additions to the theory since then. Dintzis has proposed a novel method for specific, quantized stimulation of the immune response, known as the immunon theory. The model that was developed in this investigation is based on the clonal selection theory and Bell's overall kinetic scheme. Dintzis's theory is merged into the Bell framework and the immunon concept is developed further with an equilibrium step dependent upon antigen concentration between the two paths the immune response can follow after target cell stimulation into proliferating cells. All of the events are modeled in terms of coupled kinetic equations which are solved by standard numerical integration methods using stiff differential equation subroutines. The new model also accounts for the characteristics of the immune response: specificity, recognition, memory, and low/high dose tolerance. The model is flexible enough to investigate many of the current problems and paradoxes in theoretical immunology.



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"A Generalized Kinetic Model of the T-cell Independent Primary Immune Response"

A Trident Scholar Project Report

by

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Accepted for Trident Scholar Committee

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Abstract

A generalized kinetic model has been developed which describes the T-cell independent antibody-mediated primary immune response. Immunology is a very young science and its history is important to understand the direction of investigation of those questions which remain unsolved. The immune system itself, with all the cells, cellular products, and lymph system, is very complex, second only to the nervous system for complexity in the human body. The original theoretical kinetic model was developed by Bell in 1970. However there have been many additions to the theory since then. Dintzis has proposed a novel method for specific, quantized stimulation of the immune response, known as the immunon theory. The model that was developed in this investigation is based on the clonal selection theory and Bell's overall kinetic scheme. Dintzis's theory is merged into the Bell framework and the immunon concept is developed further with an equilibrium step dependent upon antigen concentration between the two paths the immune response can follow after target cell stimulation into proliferating cells. All of the events are modeled in terms of coupled kinetic equations which are solved by standard numerical integration methods using stiff differential equation subroutines. The new model also accounts for the characteristics of the immune response: specificity, recognition, memory, and low/high dose tolerance. The model is flexible enough to investigate many of the current problems and paradoxes in theoretical immunology.

Preface

This work is dedicated to advancing the field of immunology in hopes of finding a therapy for cancer.

Table of Contents

I. Introduction

A. Background

There has been a great deal of recent interest in theoretical aspects of biology and biochemistry with extensive investigation occurring in a variety of areas. For example, there has been extensive mathematical modelling of enzyme reactions [1], (which was the original proposal for this investigation), ecological studies and population dynamics [2], kinetics of the transformation of normal cells into cancerous cells [3], and kinetic modelling of the immune response [4,5,6]. It was originally proposed for this investigation that a study of the kinetics of oscillatory biochemical enzyme reactions be attempted. Subsequent to our initial work in modelling the enzymatic breakdown of glucose [7], our interest turned to the problem of developing a kinetic model of the immune response.

B. Organization

A theoretical mathematical model of the kinetics of the human body's primary immune response has been developed. To simplify the presentation of the results obtained, the following organization will be followed. First, section II includes a brief definition

of the science of immunology. In section III, the historical development of the science of immunology will be discussed. By appreciating the history of immunology (and especially theoretical immunology), one can then take on the monumental task of understanding the immune response in detail. In section IV, a detailed description of the characteristics of the immune system will be discussed, along with a detailed description of its basic mechanism. The model that has been developed during this investigation is based on a theoretical model of the primary immune response published by Bell in 1970 [4]. In section V, a detailed description and critical discussion of Bell's work will be presented. Subsequent studies of the immune response have been performed by Dintzis et al. [8], including development of a theoretical model of the primary immune response which focuses on recognition of foreign invaders and the mechanism of stimulating the immune response. In section VI, a detailed description and critical discussion of Dintzis' work will be presented. The theoretical model developed and analyzed in this investigation uses Bell's work as a basic framework, but extends his work by including ideas from some of the more recent theories on recognition and stimulation of the immune response, such as Dintzis' work. In section VII, the theoretical model developed in this

investigation will be presented, including the incorporation of Dintzis' and Bell's concepts as well as a critical discussion of the model. In section VIII, the computational data supporting the model's validity will be presented, analyzed, and discussed. The generalized kinetic model of the T-cell independent primary immune response developed during this investigation has much potential for future expansion to possibly address some of the current problems and paradoxes in theoretical immunology [9]. In section IX, future applications of this model will be presented. Finally, a brief summary will be presented in section X.

V. Discussion of Bell's Kinetic Model

A. Introduction and Description

Bell [4] published a model in 1970 based on the clonal selection theory which describes the kinetics of the antibody-mediated (i.e., T-cell independent) primary immune response. A basic schematic of Bell's model is shown in figure 7. Bell's model has target cells stimulated by antigen to become proliferating cells that self-replicate and produce antibody. When the antigen stimulation decreases, or the concentration of antigen goes down, the proliferating cells cease production of antibody and asymmetrically divide to become terminal plasma cells and memory cells [4].

B. Assumptions and Hypotheses

As with nearly any theoretical model, there are several assumptions and hypotheses which must be described. First, nearly all of Bell's quantitative assumptions are based on experimental data. Based on experimental observations of various antigen/antibody reactions, Bell deduces that there is a certain affinity quotient (i.e., equilibrium constant) for the interaction between antigen and receptor site on a particular target cell. Bell says that the average number of bound

cells so that the next time the body is exposed to that antigen, the secondary response, which is much more rapid and powerful, is initiated [4].

and memory cells.

The plasma cells are terminal cells. A schematic (figure 6) of the immune system shows a target cell being attacked by antigen which is instantly transformed into a proliferating cell which divides asymmetrically to form the memory cell and the plasma cell. The memory cell remains in the blood plasma to be available for generating the secondary response if the body is exposed to that particular antigen again [26].

C. Primary versus Secondary Antibody-Mediated Immune Response

The primary response is the reaction to the body's first exposure to a particular antigen. The secondary response is the reaction after the body has been previously exposed to a particular antigen. This encompasses the concept of immunologic memory. Generally, the secondary response is much stronger due to the large numbers of memory cells which are formed during the primary response. Therefore the primary and secondary responses are very closely linked to the memory and specificity aspects of the immune system. The secondary immune response is the central concept to vaccination against foreign matter, where, for example, a flu shot stimulates the immune system to build up memory

individual cell, bacterium, virus, organic polymer, or just simply cellular debris. In the T-cell independent antibody-mediated immune response, the antigens are generally 100 times smaller than the target cells and normally several antigens are required to attack one target cell to elicit an immune response [5]. The antibodies are proteins (also known as immunoglobulins) which are much smaller than the antigens (see figure 4), and normally several antibodies attach to one antigen forming the complex that is filtered out of the blood stream (see figure 5). The antibody is formed of two amino-acid chains with part of the chains being identical (referred to as the constant domain) and one end, as Pauling hypothesized [20], being a highly variable end. The constant regions plus variable ends help account for the diversity of the immune response [25].

B. Mechanism of the Immune Response

In the general mechanism of the immune response, a resting target cell is stimulated by several of the appropriate antigens until it makes a transformation into a proliferating cell. The proliferating cell then begins replicating, or reproducing itself, at the same time producing antibody. Eventually the proliferating cells divide asymmetrically and produce terminal plasma cells

IV. Detailed Discussion of the Immune System

A. Characteristics and Description of the Immune System

The important characteristics of the immune system include its (1) specificity-- the ability to respond to a specific antigen, (2) adaptivity-- the ability to generate an immune response to essentially all antigens, and (3) memory-- its ability to remember a specific antigen so as to give a much enhanced immune response at a later time [24].

The immune system response involves cells known as target cells, several intermediate cells, as well as antigens and antibodies. There are four types of cells. First is the target cell, which is a cell capable of being stimulated by antigen to produce proliferating cells. When stimulated by the correct antigen, the target cell transforms into proliferating cells. The proliferating cells divide asymmetrically, or into two non-equal parts, producing memory and plasma cells. The memory cells are similar to target cells and are responsible for the secondary immune response. The plasma cells are terminal cells that only produce antibody and die shortly thereafter. The antigens are the foreign bodies capable of instigating an immune response. Antigen can take many forms, such as an

to produce antibody specific for that antigen. The target cell would proliferate and produce what came to be known as a clone of antibodies. The clonal selection theory is in wide use today. However, it still has shortcomings, as it cannot fully explain some of the controversial questions which are currently being debated in the field of theoretical immunology [22].

C. Controversies and Gaps in Theoretical Immunology Today

There are many controversies and gaps in the understanding of immunology today. Among the perplexing questions are the following: (1) How does the body know how to produce such a diversity of antibodies to attack the thousands of different antigens, yet not attack its own cells and tissues? (This concept is known as self vs. non-self discrimination.) (2) How is the immune system signalled to respond? (3) How do target cells recognize the correct antigen [23]? Answers to these questions would greatly increase our understanding of why the immune system does not form a response against cancer cells and, more importantly, of how to induce a successful immune response against cancer.

there existed a huge number of target cells in the blood stream, specific for each type of antigen. However, Jerne did not say that the antigen instructed the target cell to make complementary antibody. Instead, he simply hypothesized that there was a huge number of target cells, one for each type of antigen, and that the antigen selected the correct target cell to produce complementary antibody. The natural selection theory addressed the concept of the secondary immunologic response by saying that when the antigen contacted the antibody, they formed a complex, which was later consumed by phagocytic cells, i.e., cells that eat and destroy other cells, and that the phagocytic cells stimulated other target cells to produce a large amount of antibody specific for that antigen. Jerne's theory was a selective theory, like Ehrlich's side chain theory, as opposed to the instructive theory of Pauling; however, the natural selection theory could not yet account for immunologic memory [21].

Finally, in 1957, Burnet proposed the clonal selection theory, which is still the most widely accepted theory today. Burnet assumed that each target cell had only one type of receptor on its surface and that it produced only one specific type of antibody. Therefore the antigen selected only its complementary target cell

cell big enough to have that many different receptors on its surface. Therefore, Ehrlich's model was abandoned, in anticipation of a theory that could explain how the immune system could respond to so many different antigens. Such a theory was advanced by Pauling and is known as the instructive theory of immunology [20].

Pauling's instructive theory (see figure 3a), included the postulate that there was only one type of target cell. This was based on the observation that the chemical structure of all antibodies were basically identical, with the exception of one end. Pauling said that this special end of the antibody could be changed or instructed by the target cell to complement each particular antigen. Since this end of the antibody molecule could possess essentially an infinite number of different three-dimensional conformations, Pauling hypothesized that the antigen could serve as a template, instructing the target cell how to conform the variable end of the antibodies to complement each antigen. While Pauling's instructional model successfully explained how a target cell could respond to virtually all antigens, it still offered little explanation for the concept of immunologic memory [21].

In 1955, Jerne proposed the natural selection theory of antibody formation (see figure 3b). Jerne said

girl recovered from diptheria within hours. For Behring's work he was awarded the first Nobel prize in medicine and the modern science of immunology was born [19].

B. History of Immunology after 1900

With the birth of the modern science of immunology, scientists began inquiring into the theory of how the immune system functions. An early influential theory was proposed by Ehrlich [20] in 1900, which had a lasting influence for nearly 20 years. Ehrlich's theory attempted to account for how an animal acquired immunity or immunologic memory to a pathogenic virus or bacteria. Ehrlich's theory is known as the side chain selection theory (see figure 2). Ehrlich postulated that each cell capable of producing an immunologic reponse, hereafter known as a target cell, had on its surface a number of receptors for each particular antigen with which it might come in contact. When a particular antigen contacted any one of the target cells, the receptor site that the antigen touched would signal the target cell to begin producing antibodies, complementary to that particular antigen. This theory was widely accepted until the 1920's when scientists began to realize the magnitude of the number of possible antigens, and that there was no

saved more lives in the past 200 years than any other scientific discovery. Unfortunately, Jenner's discovery had little impact on preventing any other disease or on our detailed understanding of the immune system for at least another 100 years. Ehrlich, a prominent immunologist in the early 1900's, made the following statement [18] regarding the lack of progress after Jenner: "That Jenner's discovery remained so isolated was due essentially to the fact that the theoretical conceptions of the cause and nature of infectious diseases made no advance during the subsequent decades." In other words, it wasn't until infectious diseases were actually understood that medicine could take advantage of the immune system and immunize or vaccinate people against other infectious diseases.

In 1889, Behring studied the immunization of mice against diptheria. Behring would inject rabbits with the toxoid causing diptheria and then take a small sample of the infected rabbit's blood serum and inject it into healthly mice. When the mice were subsequently exposed to the diptheria toxin they did not become ill. Thus the rabbit's blood serum had transferred immunity towards diptheria to the mice. In 1890, a young girl was dying from diphtheria and as a final measure, the blood serum from an infected rabbit was injected into the girl. The

III. A Brief History of Immunology

A. History of Immunology before 1900

The science of immunology gets its name from the Latin word Immunity, where in ancient Rome, a person who had immunity was exempt from service or duty to the state. The concept of immunity was first recorded in about 430 B.C. by Thucydides [16] who made the following observation during the plague of Athens:

> Yet it was with those who had recovered from the disease that the sick and the dying found most compassion...(for they) had no fear for themselves...for the same man was never attacked twice-- never at least fatally.

It wasn't until many centuries later that man learned to use immunity to confront disease. In the 17th and 18th centuries smallpox was one of the deadliest diseases known. However, in 1798, Jenner [17] demonstrated that by injecting into a healthy person a small amount of pus from a cow or person infected with cowpox or smallpox, that person would be immune to smallpox. This discovery by Jenner is reported to have

figure 1b, the T-helper mediated B-cell immune response is illustrated. In this system, a T-cell is contacted by antigen. Then this T-cell, known as a T-helper cell, stimulates B-cells to produce antibodies, specific to the antigen that contacted the T-helper cell. In figure 1c, the T-cell independent B-cell immune response is illustrated. In this system, the antigen contacts the resting B-cell, stimulating it to produce antibodies specific to the attacking antigen. The B-cell immune response is the more primitive of the two and is the body's main defense against pathogenic bacteria and viruses [14]. It is also responsible for the response against polymers with chemical groups attached which have antigenic properties [15]. All of the theories and models that have been studied and developed during this investigation describe only the T-cell independent B-cell immune response.

differentiate or mature in the thymus gland to become fully effective. The T-cell immune response is characterized by the production of cytotoxic cells, or killer cells, and is the immune response most often associated with skin graft and donor organ rejection [11]. In figure 1a, the T-cell response is dramatically depicted, showing how the antigen contacts the resting T-cell, stimulating it to seek out and destroy antigens. The T-cell response is the more complicated immune response and is more advanced in an evolutionary sense [12].

The other class of immune response is the B-cell, or the humoral or antibody-mediated immune response (see figures 1b and 1c). It is associated with B-type white blood cells, or B-cells. The B-cells originate in the bone marrow, just like the T-cells. However, in certain vertebrates such as the chicken, the B-cell lymphocytes are not mature or fully functional until they have migrated to an organ known as the Bursa of Fabricus. Hence lymphocytes associated with the antibody-mediated immune response are known as B-cells. It should be noted that in the human being, which has no Bursa of Fabricus, the B-cells originate in the bone marrow but are believed to also differentiate or mature there [13].

There are two types of B-cell immune response. In

II. Definition of Immunology

A. Basic System

The immune system is the body's defense system. Its function is to recognize and destroy foreign substances, known as antigens, that invade the body. This immunologic response is mounted by the production of cells and protein-molecules, known as antibodies, which attach to and attack the antigens, subsequently removing them from the blood stream and rendering them harmless to the body. All immunologic cells and antibodies are derived from white blood cells, or lymphocytes, that originate in the bone marrow. After the immune response has incapacitated the invading antigens, the lymphatic system, through its network of lymph nodes, lymph ducts, and the spleen, filters the antigen-antibody complexes out of the blood, eliminating the antigens from the body [10].

B. Classes of Immune Response

There are two broad classes of the immune response. One type is the T-cell or the cell-mediated immune response (see figure 1a). This system makes use of T-type white blood cells, also referred to simply as T-cells. The "T" refers to the fact that T-cells must

receptors on a target cell determines how quickly it will transform into a proliferating cell, and relates the average number of bound receptors to the rate of the transformation of a target cell into a proliferating cell.

C. Results

Bell [4] has compared the results of his model with empirical data, thereby attempting to account for each step in his mechanism. He has calculated the time-dependence of the various species on the computer using differential equation solving techniques and has produced a graph of the concentrations of the cells versus time. The rate constants he used were based on experimental data. The graph shows the concentration of each cell as a function of time [4]. There is a relatively constant antigen concentration until the point has been reached where the antibody concentration has built up high enough to effectively attack it, at which point the memory cells and plasma cells are formed and the proliferating cells decrease.

D. Discussion and Conclusion

A significant aspect of Bell's model is that it is not a purely kinetic model. He makes use of "sliding"

rate constants, which are rate constants that change their values during the course of the response, (hence not actually constants), depending upon relative concentrations of various intermediates. As a consequence of these features, Bell's model gives an accurate description of the kinetics of the primary immune response, accounting for the concepts of low/high dose tolerance (i.e., the phenomenon in which there is no immune reponse for an excessively low or high dose of antigen), specificity, and memory. It would be very useful to develop a model which does not make use of sliding rate constants, i.e., a model in which the rate constants are the same values no matter what the concentrations of the intermediates.

VI. Discussion of Dintzis' Model

A. Background

In 1982, Dintzis [8] published a theoretical model of the immune system focusing of the transformation of a target cell into a proliferating cell. Dintzis' work is based entirely on equilibrium analysis as opposed to kinetic analysis, and is well supported by experimental data. Dintzis' work addresses solely the recognition, stimulation and transformation of the target cell into a proliferating cell, and he discusses in detail the interactions between a target cell and an antigen on a cellular and macromolecular level.

B. Description

Dintzis describes a synthetic linear polymeric antigen with a specific number of antigenic determinants (or haptens) per molecule. An example of these hapten groups is the dinitrophenyl group (see figure 8) that is known to react with the receptors on the target cell membrane. When an antigen with haptens in its structure contacts a target cell, the haptens associate with the receptors on the target cell membrane, forming bound hapten/receptor site complexes. Dintzis claims and proves experimentally that a certain quantized number of

these haptens must be bound to stimulate a target cell into becoming a proliferating cell. When this specific quantized number of haptens has been formed, a fundamental transformation takes place on the surface of the target cell. The bound haptens cluster together forming what Dintzis calls an "immunon" (see figure 9). It is the formation and presence of a number of these immunons, according to Dintzis, which signals a target cell to transform into a proliferating cell. In other words, the immunon is defined as the "device" that actually "triggers" or signals the target cell into becoming a proliferating cell which subsequently can produce antibody.

C. Discussion

This requirement for immunon formation addresses several of the controversial questions being debated in theoretical immunology, such as the concepts of low/high dose immunologic tolerance and antigen specificity. The immunon model explains the case of low dose immunologic tolerance. In that case the antigen concentration is not high enough to support the formation of enough immunons per cell to stimulate target cells into becoming antibody-producing proliferating cells. On the other hand, if there is too much antigen, high dose immunologic

tolerance would be observed, because the antigen concentration is so high that the antigens compete vigorously for receptors on the target cell's surface. With this competition for the limited number of receptor areas on a target cell, the quantized number of bound haptens is never reached (i.e., the immunon is never formed), and proliferating cells are not produced. This concept of the immunon not forming under high antigen dose conditions is supported by theoretical work done by Waite [27].

This immunon concept also addresses the notion of antigen specificity. If the antigen does not have enough hapten determinants, it cannot possibly form enough bound hapten groups, and the immunon transformation on the membrane of a particular target cell cannot occur. Therefore the target cell is not capable of transforming into a proliferating cell, no antibody is produced, and there is no immune response. Likewise, if an antigen has too many of these antigenic determinants on its surface, an immunon will not form and there will also be no immune response. Dintzis believes this quantized number to be approximately 15-20 for the case where the DNP (i.e., dinitrophenyl) groups are attached to the monomeric DNO-lysine (a large protein carrier molecule). If another target cell, one that is specific for that

antigen, comes into contact with that antigen, and the quantized number of haptens is formed, the target cell will form immunons and will be transformed into a proliferating cell, assuming the concentration of antigen is within tolerance limits, and antibody production will commence.

VII. The Generalized Kinetic Model

A. Introduction and Background

We have taken Bell's [4] basic model, including Dintzis' [8] ideas, and incorporated modifications in order to produce a more generalized kinetic model of the primary immune response. There are still many shortcomings with this model, as will be discussed. However, it is adaptable to many of the current contradictions and paradoxes of theoretical immunology.

B. Mechanism and Description

The basic framework of the Bell model has been modified so as to be a purely kinetic model. In addition, we have incorporated and further developed the Dintzis immunon concept, resulting in a unique generalized kinetic model of the T-cell independent immune response. This model is based on the clonal selection theory, and still most closely resembles Bell's model in general features.

In figure 1Ø, a diagram of this model is shown which depicts the basic mechanism of the immune system and the resemblance to Bell's model. The target cells are all of the same clone of target cell, the only difference being that they have zero, ten, twenty, and thirty bound

haptens formed. It can be seen from the diagram that there is a particular (i.e., quantized) number of haptens that must form for the target cell to become a proliferating cell as dictated by the Dintzis immunon concept. Of the four different configurations of the one clone of target cell shown, only the target cell with 20 bound haptens formed has reached the appropriate configuration leading to immunon formation. Therefore only this target cell configuration is allowed to become a proliferating cell. Thus this model incorporates Dintzis' immunon idea in the target cell recognition of its complementary antigen as well as the concept of immunon formation to trigger a target cell into becoming a proliferating cell.

Dintzis says that the immunon must be formed and therefore is simply a permanent switch on or off for the target cell to become an active proliferating cell, dividing and producing antibody. However, Dintzis' immunon idea is developed more thoroughly in the model described in this investigation. The new model treats the immunon as being actually in equilibrium with the existing, free concentration of antigen and that the production of antibody by proliferating cell is controlled indirectly by the local free concentration of antigen. In this model, the antigen concentration must

be between certain limits for antibody production to occur. This model incoporates a Le Chatelier type equilibrium process between activated proliferating cell, non-activated proliferating cell, and antigen.

The direction that the equilibrium is shifted will control which path the immune response will follow, and subsequently whether antibody and memory cells will form. If the antigen concentration is low, the equilibrium between the activated proliferating cell and the non-activated proliferating cell will be shifted to the right in the direction of the non-activated proliferating cell, with little production of antibody and full-scale production of memory cells and terminal plasma cells. If the antigen concentration is high, the equilibrium will be shifted in the direction of the activated proliferating cell, which will continue replicating and producing antibody. The antibody produced reacts with the antigen, forming an antigen-antibody complex. The formation and elimination of the antigen-antibody complex decreases the amount of free antigen, which will shift the equilibrium in the direction of the non-activated proliferating cell. Therefore when the antigen concentration is high (as is the case during initial exposure), the antigen concentration stays high while the immune system is gearing up, since there is little

initial production of antibody. When the immune system has been "switched on" and is capable of producing antibody, which forms the antibody-antigen complex, the elimination of antigen leads to a shift in equilibrium towards the non-activated proliferating cell, decreasing the production of antibody. The immunon concept of Dintzis et al. has, in a sense, been extended into the proliferation stage of the immune response, instead of just the triggering stage. Therefore this equilibrium step between the non-activated and activated proliferating cells is a further development of the Dintzis immunon concept.

C. Implication and Conclusions

The concept of the immunon has been incorporated into Bell's model and has been developed more fully and completely. The improved mechanism also addresses the concept of low/high dose tolerance in a different manner than the Dintzis model. The new model allows for high dose tolerance in much the same way as does Dintzis' immunon. If the concentration of antigen is so high that immunons cannot form (due to the intense competition for the receptor sites on the target cell's surface), there will be no immunons formed and no immune response. This model explains low dose tolerance similar to Bell's

model. The new model would allow a very low concentration of antigen to still form immunons. The formation of immunons under low concentration is in direct contrast to what Dintzis claims. The new model allows for low dose tolerance where, if the concentration of antigen is low, the Le Chatelier equlibrium between the activated proliferating cell and non-activated proliferating cell would be shifted in the direction of the production of memory cells, terminal plasma cells, with little or no production of antibody.

Finally, the new model is purely kinetic, meaning that the rate constants do not change with time or with changing concentrations of the intermediates. In Bell's kinetic model, he explains immune recognition, stimulation and low/high dose tolerance by changing the values of his rate constants. The model developed in this investigation is purely kinetic and explains recognition, stimulation, and tolerance in the basic mechanism of the model. This fundamental kinetic mechanism, rather than the incorporation of variable "constant" parameters, lends more credibility to the model's explanations for certain characteristics and observations of the immune response.

VIII. Discussion of Results

A. Description of Method of Computation

Based on the mechanism shown in figure 10, each species has associated with it a first order differential equation describing its rate of production or consumption. For example, in the transformation of a non-activated proliferating cell into a memory cell (see figure 10), the rate is expressed by:

$$d(SM)/dt = SN1*(BP1)$$

For the explanation of notation, see tables accompanying figure 10. Another more complicated example is the rate of antibody change, given by:

$$d(AB)/dt = TR2*(PL) - Q1*(AB)*(SL) + Q1M*(ABL) + SK1*(BP1A)$$

All of the other kinetic equations are given in the table accompanying figure 10. By assigning a first order differential equation to each event in figure 10, a system of differential equations is obtained, which can be solved on the digital computer. The Naval Academy Time Sharing system (NATS) was used for the computations, programming being done in Fortran F84 The International Mathematics and Science Library (IMSL) subroutine "DGEAR," which is capable of solving systems of

first-order differential equations, was used. A data file was generated of the concentrations of each of the cells in the immune respone as a function of time. From this data file, plots were obtained using the TEKGRAF3 plotting software on the Tektronix plotter and a graph was generated of the cell's concentrations as functions of time. A representative example of this plot is shown in figure 11.

B. Discussion of the Plot

This plot, figure 11, displays the kinetics of the primary immune response. Upon initial inception of the foreign invader into the body, the antigen concentration remains high for a long time period until the antibody concentration can rise up high enough to affect it. When the antibody concentration is sufficiently high enough, a sharp decrease occurs in the antigen concentration. This moment in time where the antibody concentration surpasses the antigen concentration is the "break-point". On the lower portion of the graph, the concentrations are displayed of all the intermediate cell types involved in the response, e.g., activated proliferating cell, non-activated proliferating cell, plasma cells and memory cells. All of these concentrations begin at zero and rise exponentially with time until the "break-point"

occurs. At the "break-point" many events start occurring. First, the activated proliferating cells, which, prior to the "break-point" had been about one order of magnitude more concentrated than the non-activated proliferating cells, rapidly decrease in concentration until at the termination of the immune response, the non-activated proliferating cells actually outnumber the activated proliferating cells by three or more orders of magnitude. Furthermore, at the break-point the terminal plasma cells begin dying off with time and the memory cells remain at a steady, constant concentration.

C. Memory Cell Feedback

The presence of the memory cells gives rise to the secondary immune response. If figure 11 were extended another 1000 hours or so and memory cells were allowed to feed back as target cells, a new injection of the same antigen would result in a much more impressive secondary immune response. In figure 12, the feedback loop for the memory cells to become target cells is shown. When the model is run on the computer and the concentration of memory cells is added to the initial concentraton of target cells, a secondary immune response is simulated. This secondary immune response is much more rapid and

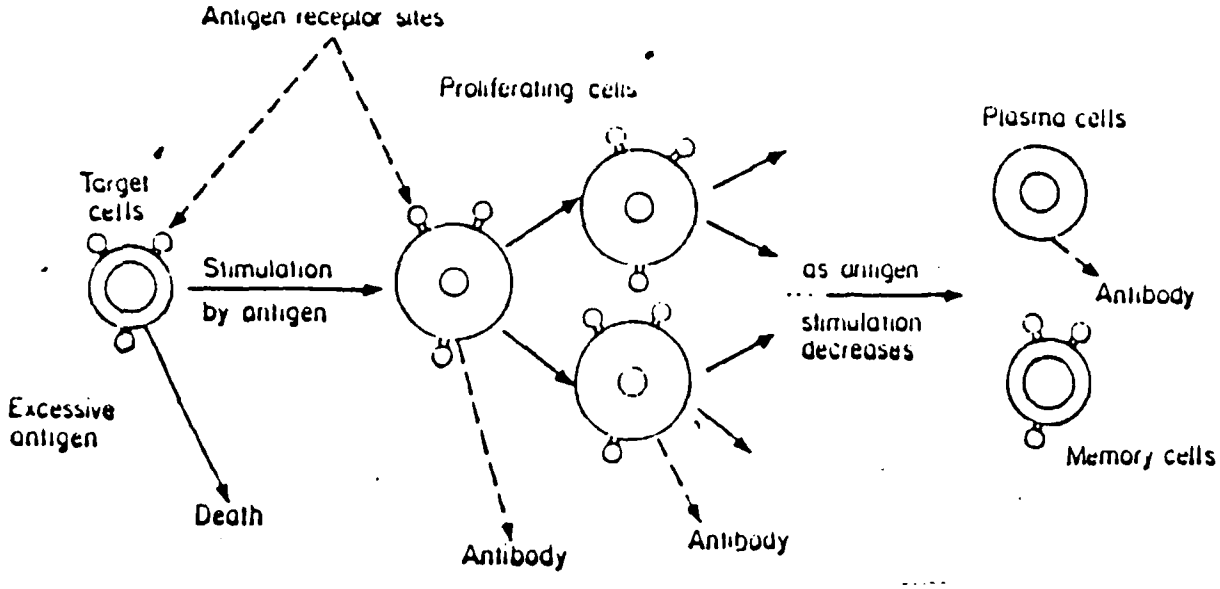


Figure 7. A Schematic of Bell's kinetic model of the T-cell independent primary immune response.

(see reference 4.)

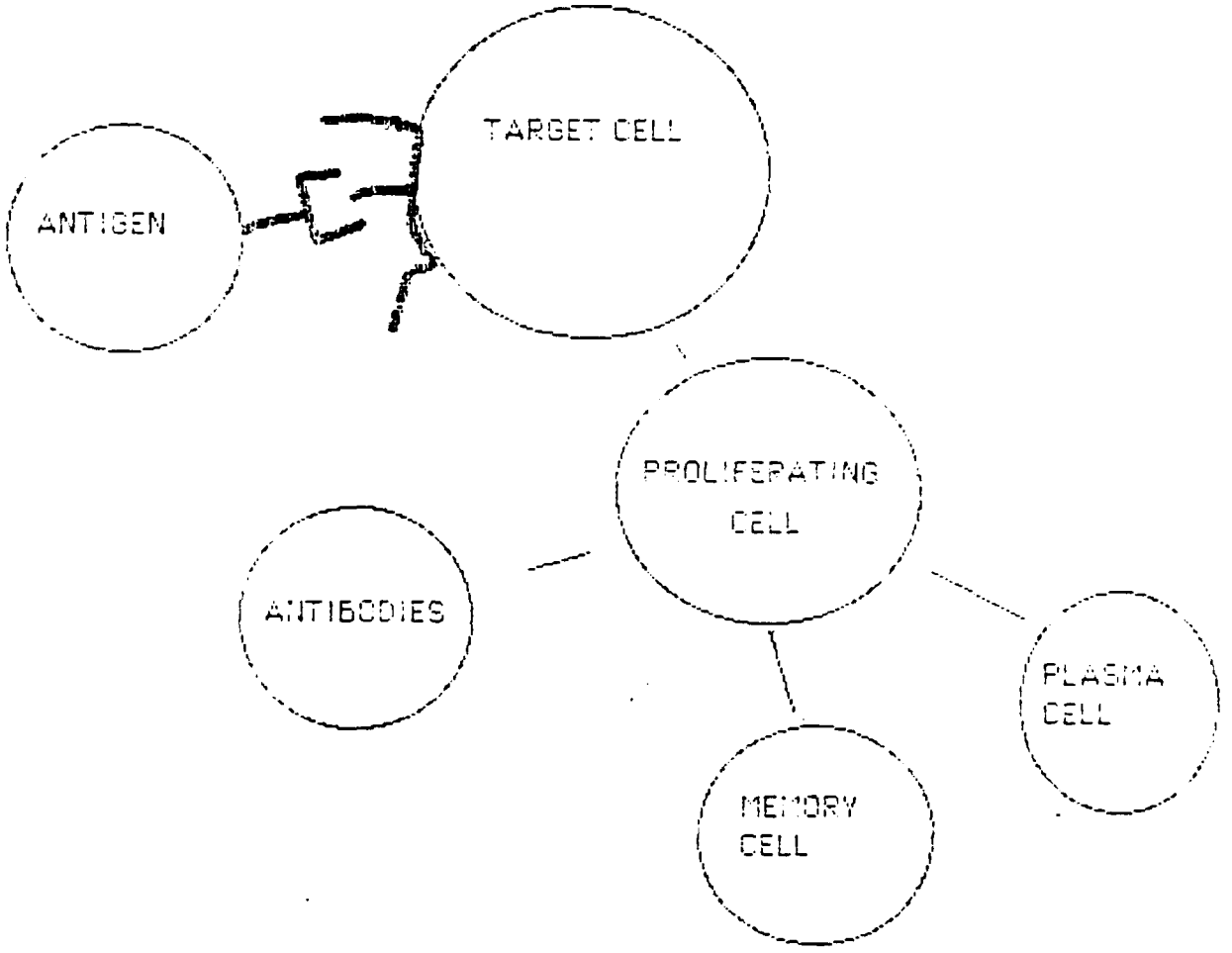


Figure 6. Basic Schematic of the T-cell independent B-cell immune response.

The antigen stimulates the B-type target cell to transform into a proliferating cell which produces antibody. Eventually the proliferating cell asymmetrically divides to produce a plasma cell and a memory cell.

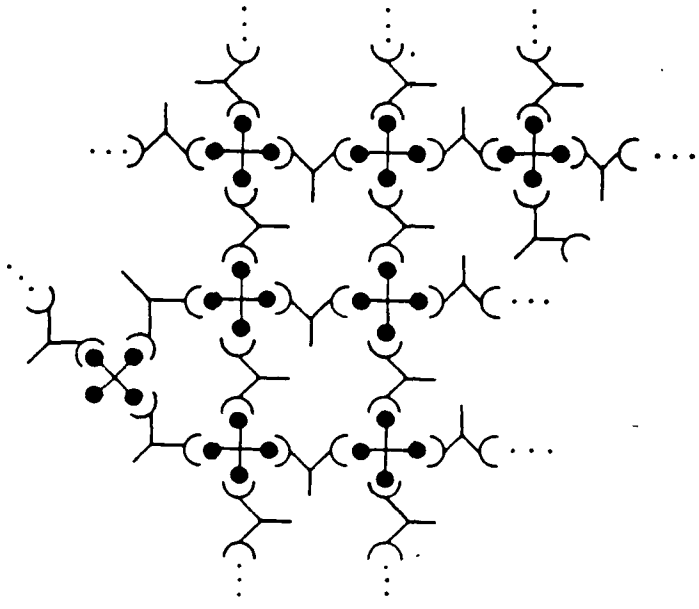


Figure 5. Diagram of an antigen-antibody complex.

The antigens and antibodies agglutinate to form complexes such as this one pictured. These complexes are filtered out of the bloodstream and eliminated from the body. The antibodies, shown as "Y"'s surround the antigens (shown as the crosses).

(from Immunology, Hood et al., 1984, p. 49.)

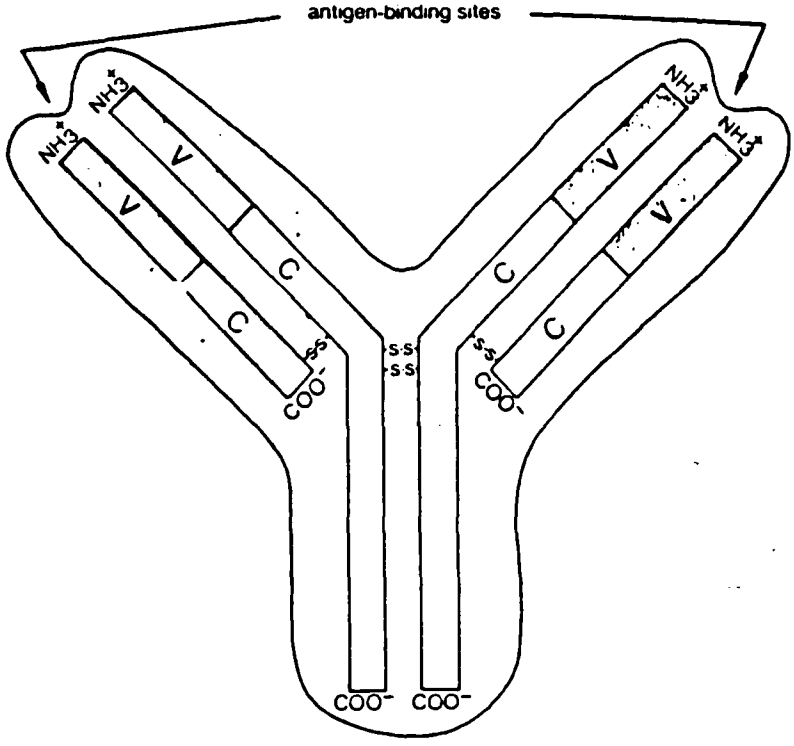


Figure 4. Diagram of an antibody.

The two tips of the Y on the antibody are the variable regions, designated here by the "V" and can vary to complement any antigen.

(from Immunology, Hood et al., 1984, p. 7.)

Fig. 3a

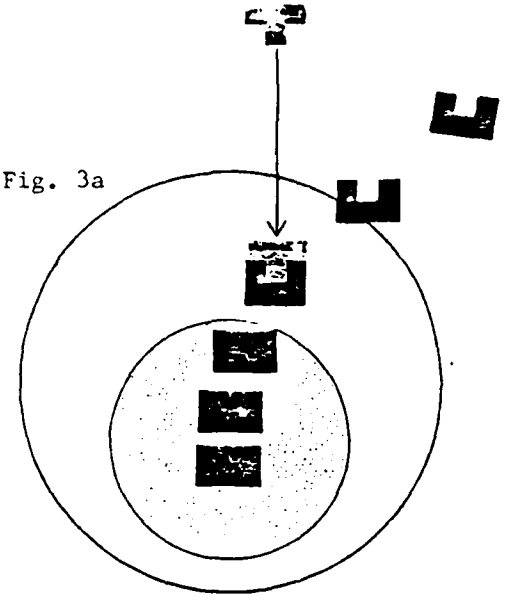
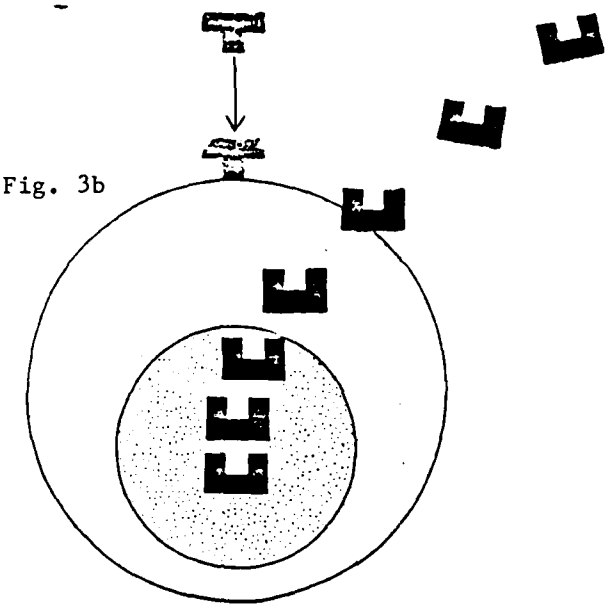


Fig. 3b



Figures 3a and 3b.

Instructive versus Selective Theories of target cell stimulation.

Figure 3a.

The instructive theory. It is hypothesized that there is only one type of target cell and it can be instructed by antigen to produce complementary antibodies.

Figure 3b.

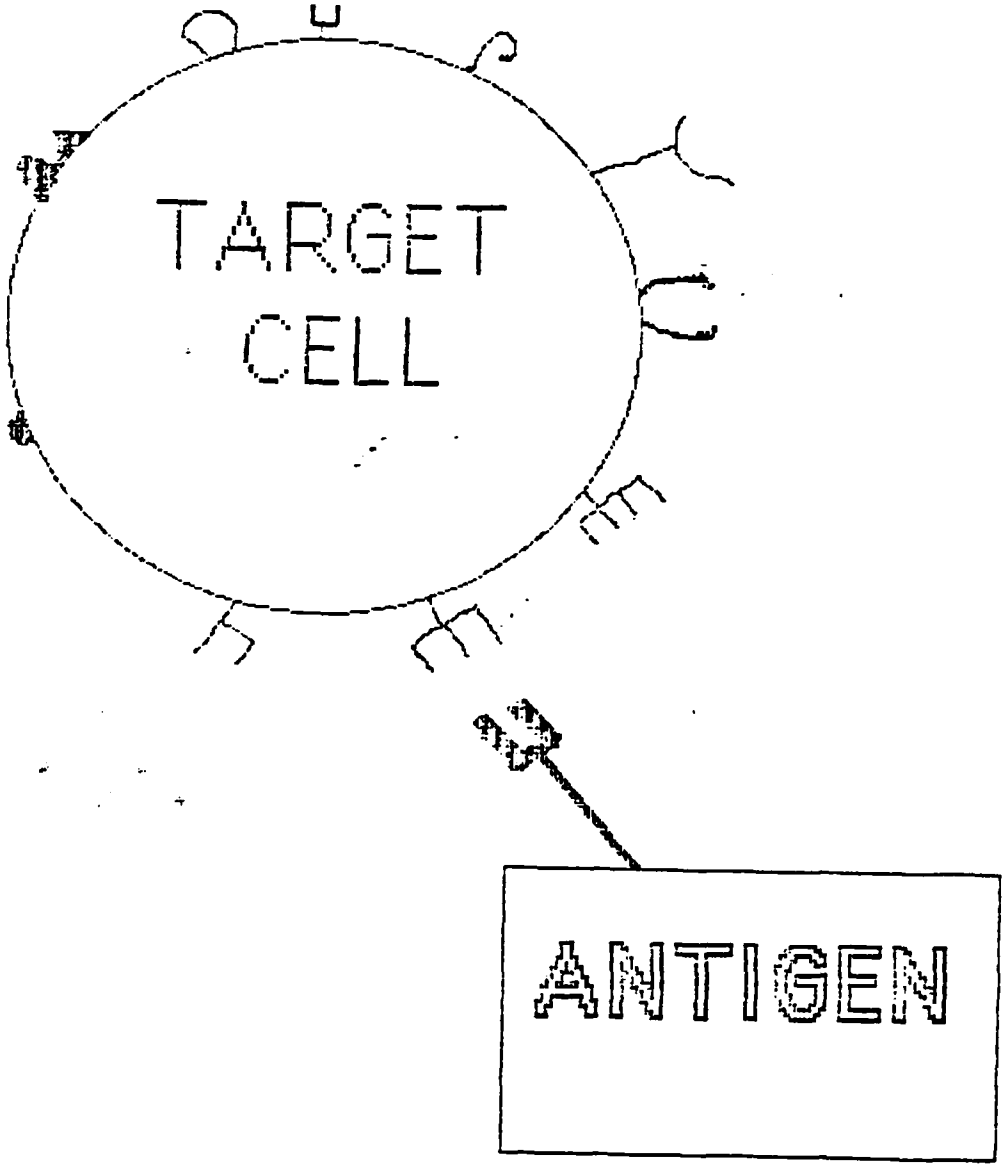
The selective theory. It is hypothesized that there are thousands of target cells and each target cell is specific for one antigen to produce one type of antibody. This theory is currently the prominent theory on target cell stimulation.

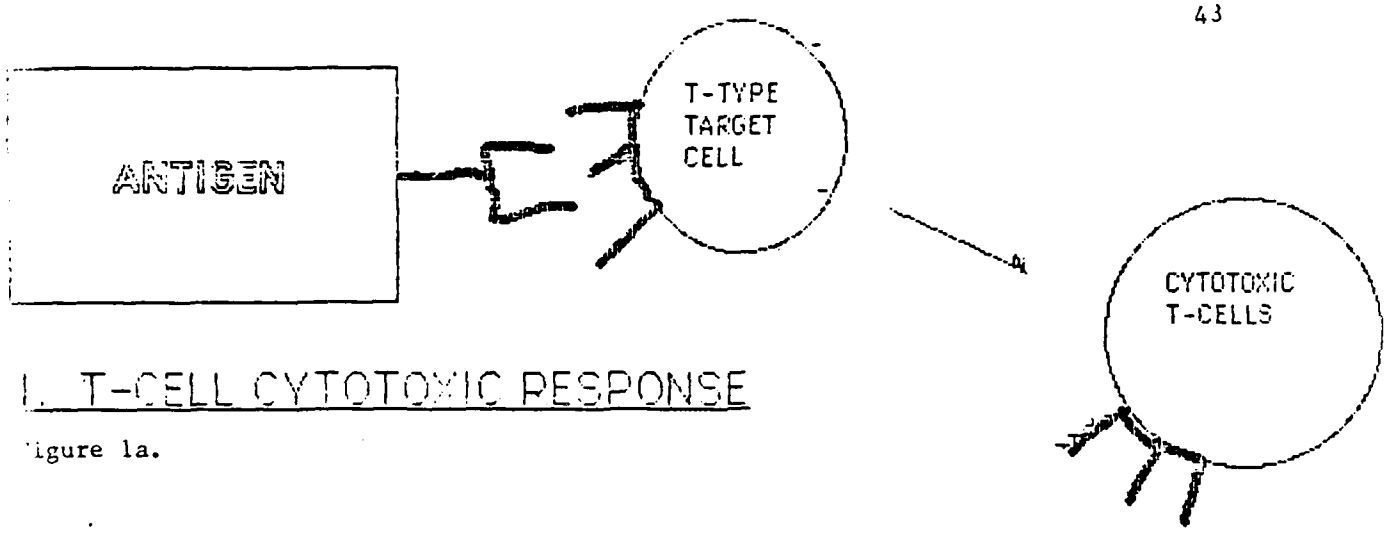
(from Readings from Scientific American, Immunology, 1976 p. 28.)

Figure 2.

Ehrlich's Side Chain Theory

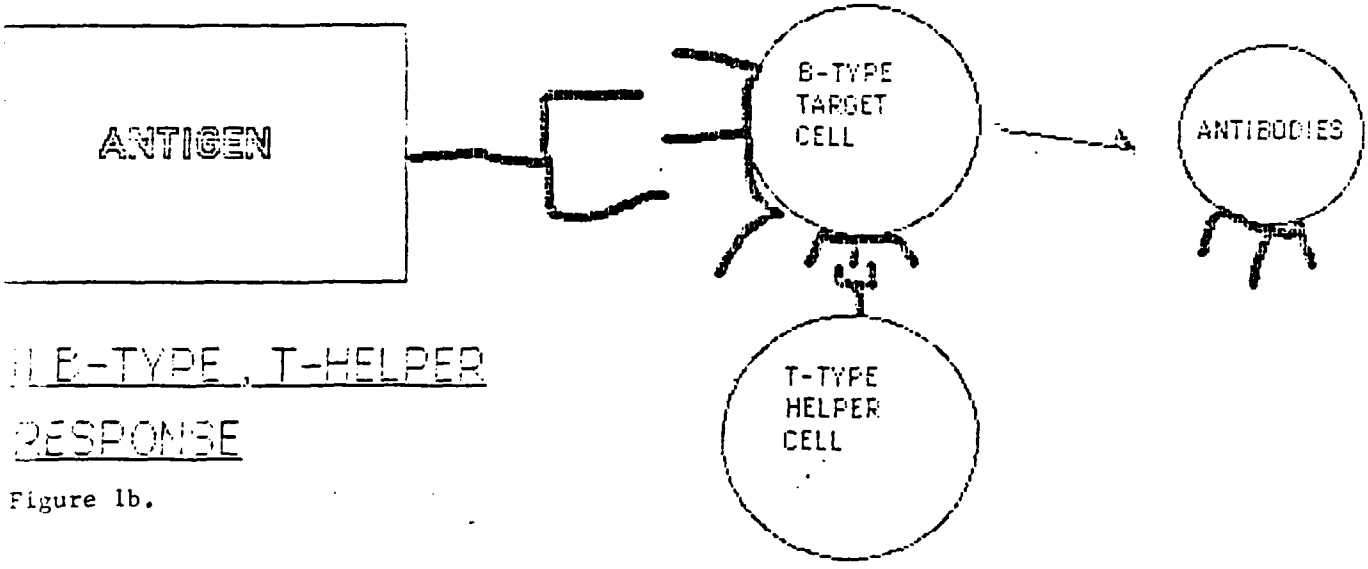
Ehrlich hypothesized that all target cells were identical and that each target cell had receptors for every type of antigen.





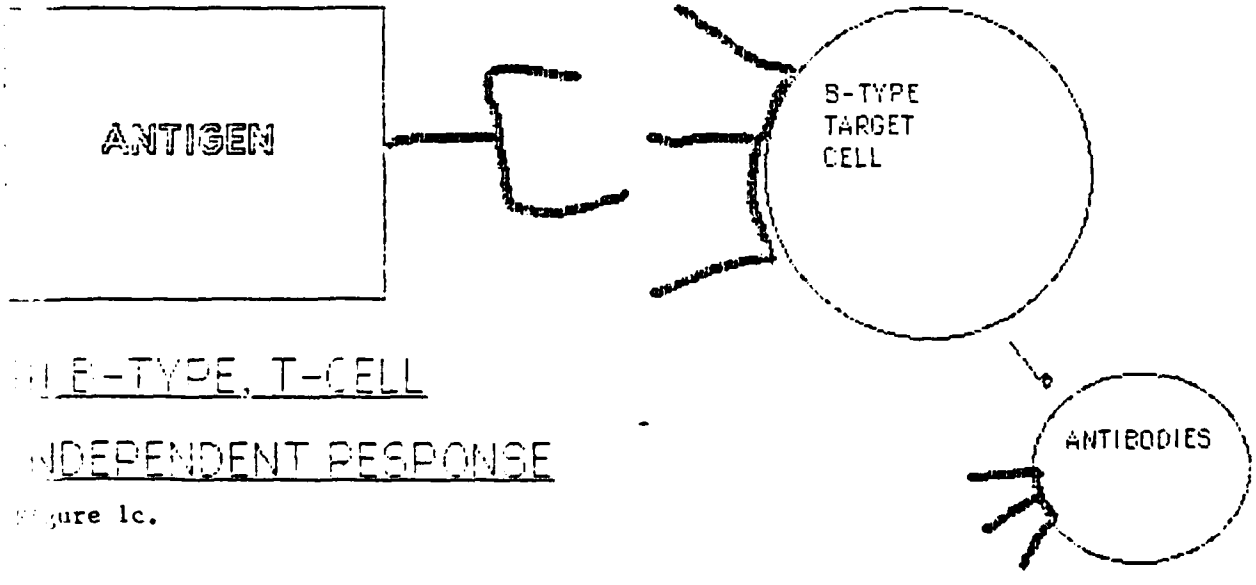
I. T-CELL CYTOTOXIC RESPONSE

Figure 1a.



II. B-TYPE, T-HELPER RESPONSE

Figure 1b.



III. B-TYPE, T-CELL INDEPENDENT RESPONSE

Figure 1c.

Explanation to accompany Figures 1a, 1b, and 1c.

Figure 1a.

This is a diagram of the T-cell Cytotoxic immune response. The antigen stimulates the T-type target cell to produce cytotoxic T-cells.

Figure 1b.

This is a diagram of the T-helper cell B-cell immune response. The antigen stimulates the B-type target cell to produce antibodies only with the assistance of a T-type helper cell.

Figure 1c.

This is a diagram of the T-cell independent B-cell immune response. The antigen stimulates the B-type target cell to produce antibodies. The kinetic model developed in this investigation models this type of immune response.

characteristics of the immune response; specificity, recognition, memory, and low/high dose tolerance.

There is considerable room for expansion with this generalized model to address some the current problems and paradoxes in theoretical immunology. However, it should be noted that a theoretical model is limited in its usefulness and is just one of the investigator's tools in attempting to understand the immune system. There is obviously a need for quality empirical data in order to compare with theoretical predictions. In this light, it is hoped that the results of this investigation have somehow expanded upon the general understanding of immunology and helped to catalyze efforts in possibly using immunology as a cancer therapy.

X. Conclusion

The problem of modelling the primary immune response has been addressed. The immune system has two broad classes of response systems: the T-cell system or cell-mediated response and the B-cell system or the humoral, or antibody, response. The T-cell independent B-cell primary immune response was investigated. The immune system itself, with all the cells, cellular products, and lymph system, is very complex, second only to the nervous system for complexity in the human body. The original theoretical kinetic model was developed by Bell [4] in 1970. However there have been many additions to the theory since then. Dintzis [8] has proposed a novel method for specific, quantized stimulation of the immune response and attempts to account for several of the characteristics of the immune response. The generalized kinetic model that was developed during this investigation is based on the clonal selection theory and Bell's overall kinetic scheme. This new model also includes the concept of the immunon and develops it further with an equilibrium step dependent upon antigen concentration between the two paths the immune resonse can follow after target cell stimulation into proliferating cell. The new model also accounts for the

clinical research. However, there are limitations to a generalized model such as this and some experimental observations and determinations simply have to be made in order to test the validity of the model.

X. Future Applications of the Generalized Model

This model is capable of much further development and the possible addressing of many of the current paradoxes in theoretical immunology. In particular, Perelson [9] has proposed several contradictions to Dintzis'immunon theory relating mostly to multi-valent antigens and antibodies. Perelson attempts to prove that clusters of bound haptens would be unlikely to form due to the close proximity of target cells and antigens where there would be much cross linking and competing reactions. Waite already has preliminary results using a theoretical model of a cell surface that show the lifetime of a cluster is inversely proportional to the size [24].

Further refinement of this model developed in this investigation may lead to an addressing of these problems, such as the immunon theory and cluster size controversy [29,30,31]. In addition, when the antigen is allowed to replicate, as in the case of cancer cells, this model may assist in developing a cancer therapy based on the immune response. Development of a theoretical model of this nature can be very useful in explaining certain empirical observations and also can be instrumental in directing the course of experimental or

feedback loop for replication of the antigen is shown. If the antigen is allowed to replicate, one would expect the immune response to be slower and take more time to effectively neutralize the antigen. In figure 15, the immune response allowing antigen-replication is displayed. As expected, it takes longer for the antigen concentration to come down since it is now capable of reproducing itself. However, if the rate of replication of the antigen is varied it is possible for the antigen concentration not to come down for longer than the life-expectancy of the organism. Since cancer cells can be considered as antigens that are capable of self-replication at very high rates, this mechanism might be able to explain why the body cannot mount an immune response against cancer.

more potent than the primary immune response (see figure 13). By allowing the memory cells formed in the primary immune response to become new target cells for a particular antigen, the population of target cells for that particular antigen has been increased to a much higher level. The secondary immune response should therefore be larger based on the fact that there are simply more target cells available to be stimulated by antigen. Figure 13 displays the secondary immune response. By comparing figure 11 and figure 13, it can be seen that the immune system responds much faster and more prominently during the secondary immune response.

This phenomenon of the secondary immune response is the key idea behind vaccinations. A person being vaccinated is actually injected with a small, non-virulent or killed dose of the virus or antigen and the body generates a primary immune reponse. Next time, if the person were exposed to that particular antigen or disease, the body would respond with the potent secondary immune reponse. Therefore this person would be immunized [28].

D. Antigen Self-Replication

This generalized model can be further modified by allowing the antigen to replicate. In figure 14, the

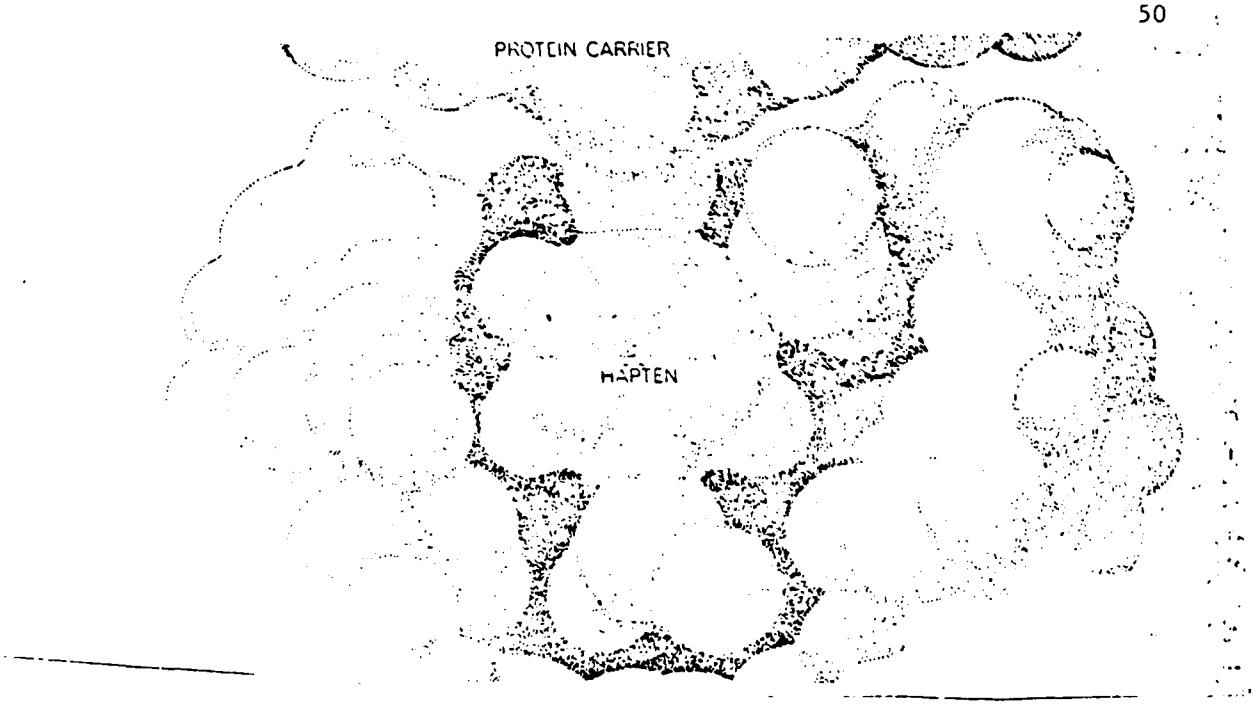


Figure 8. Diagram of the dinitrophenyl hapten.

The hapten attached to the carrier protein is a known antigenic determinant, or hapten on antigens. Here it is shown associating with its complementary receptor on the target cell surface.

(from Readings from Scientific American, Immunology, 1976, p. 40.)

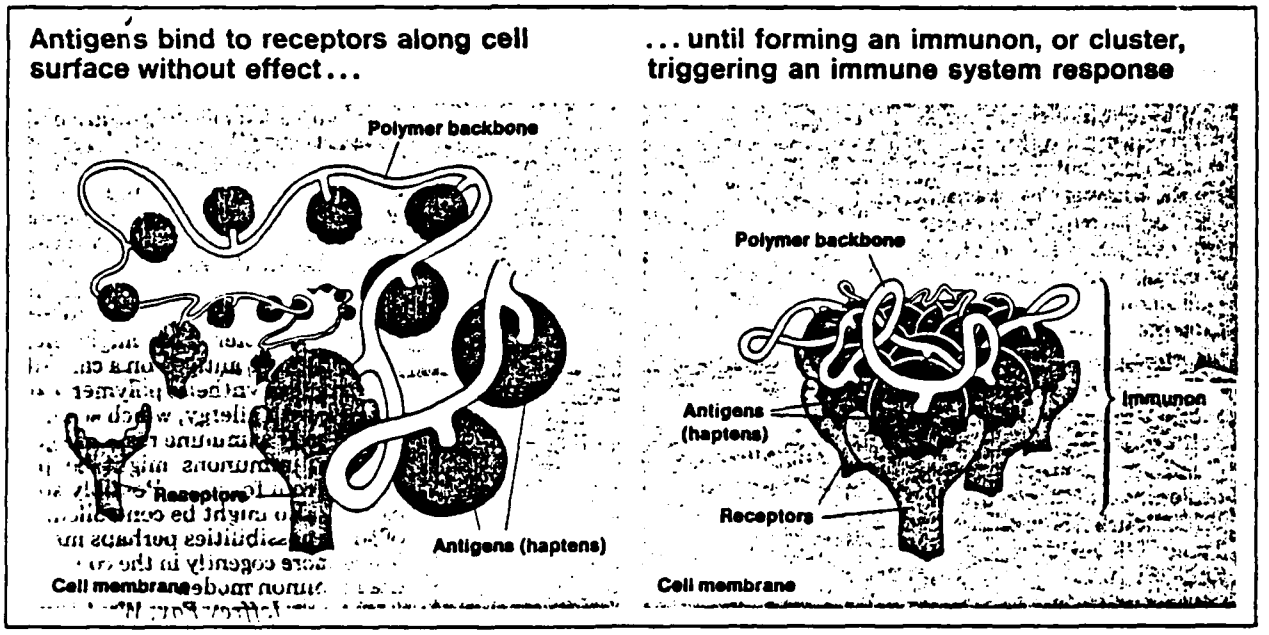


Figure 9. Dintzis' immunon concept.

Dintzis hypothesizes that when a quantized number of haptens become bound to receptors on the target cell surface, they cluster together to form immunons and trigger the immune response.

(see reference 8.)

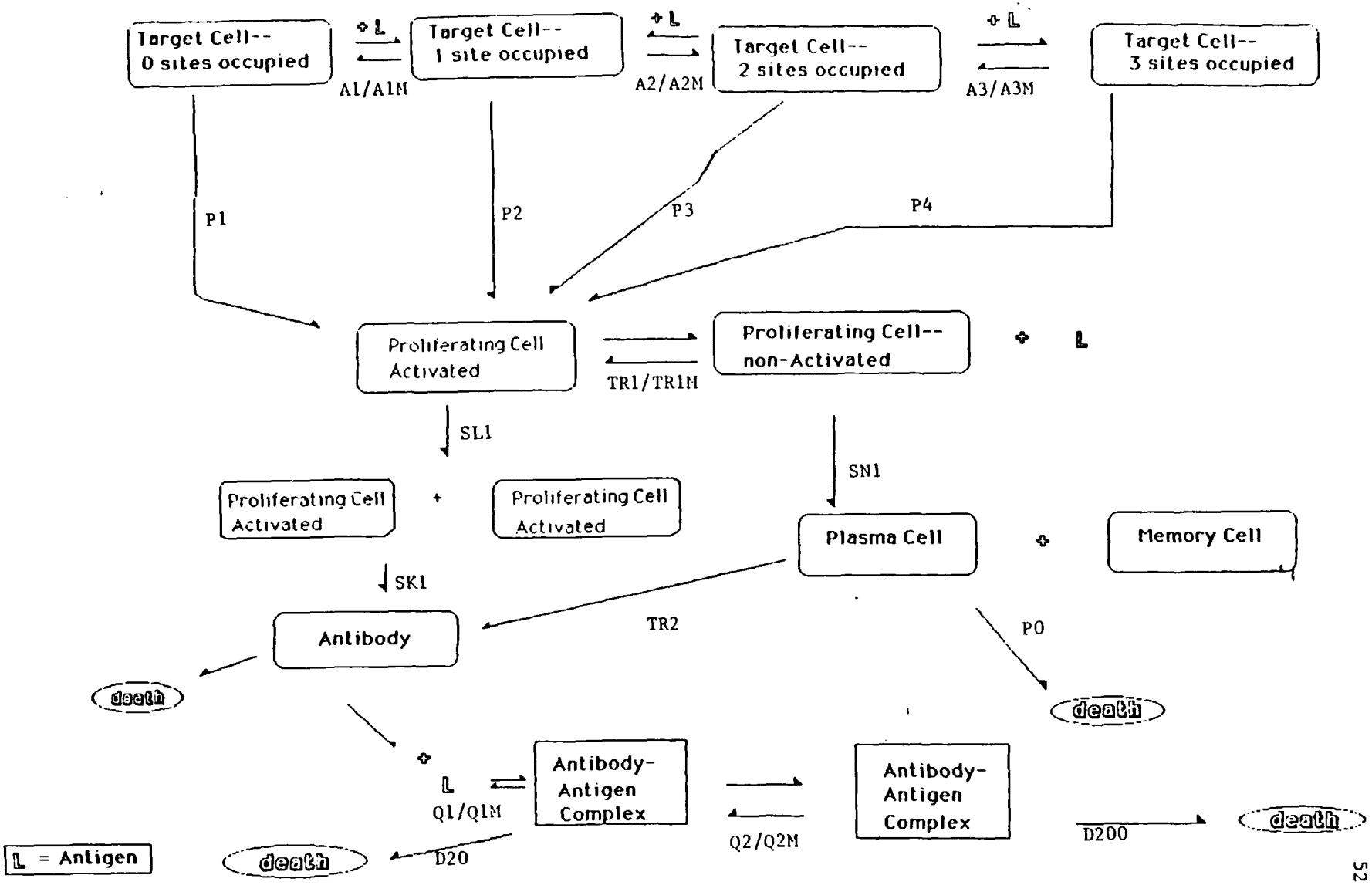


Figure 10. A Diagram of the basic T-cell independent B-type immune response that is modelled in this investigation.

A. Kinetic Equations

Legend:

T1 = Target Cell, 0 sites occupied
T2 = Target Cell, 1 sites occupied
T3 = Target Cell, 2 sites occupied
T4 = Target Cell, 3 sites occupied
BP1A = Activated Proliferating Cell
BP1 = Non-Activated Proliferating Cell
SL = Antigen
PL = Plasma Cell
SM = Memory Cell
AB = Antibody
ABL = Antigen-Antibody Complex
ABLL = Antigen-Antibody Complex (bivalent)

1. d(T1)/dt = -A1*(T1)*(SL) + A1M*(T2) - P1*(T1)

2. d(T2)/dt = -A2*(T2)*(SL) + A2M*(T3) - P2*(T2) + A1*(T1)*(SL)
   - A1M*(T2)

3. d(T3)/dt = -A3*(T3)*(SL) + A3M*(T4) - P3*(T3) + A2*(T2)*(SL)
   -A1M*(T2)

4. d(T4)/dt = A3*(T3)*(SL) - A3M*(T4) - P4*(T4)

5. d(BP1A)/dt = -TR1*(BP1A) + TR1M*(BP1)*(SL) + P1*(T1)
   +P2*(T2) + P3*(T3) + P4*(T4) + SL1*(BP1A)

6. d(BP1)/dt = TR1*(BP1A) - TR1M*(BP1)*(SL) - SN1*(BP1)

7. d(SL)/dt = -A1*(T1)*(SL) + A1M*(T2) - A2*(T2)*(SL)
   + A2M*(T3) - A3*(T3)*(SL) + A3M*(T4)
   + TR1*(BP1A) - TR1M*(BP1)*(SL)
   - Q1*(AB)*(SL) + Q1M*(ABL) - Q2*(ABL)*(SL)
   + Q2M*(ABLL) + TR3*(SL)

8. d(PL)/dt = SN1*(BP1) - P0*(PL)

9. d(SM)/dt = -TR4*(SM) + SN1*(BP1)

10. d(AB)/dt = TR2*(PL) - Q1*(AB)*(SL) + Q1M*(ABL) + SK1*(BP1A)

11. d(ABL)/dt = Q1*(AB)*(SL) - Q1M*(ABL) - Q2*(ABL)*(SL)
    + Q2M*(ABLL) - D20*(ABL)

12. d(ABLL)/dt = Q2*(ABL)*(SL) - Q2M*(ABLL) - D200*(ABLL)

B. Rate Constants

Name	Rate Constant	
A1	.1E6	
A1M	.1	
A2	.1E6	
A2M	.1	
A3	.1E6	
A3M	.1	
P1	Ø.	
P2	Ø.	
P3	.1	
P4	Ø.	
TR1	.1	
TR1M	.1E6	
SL1	.4E-1	
SN1	.1E-1	
SK1	.1E8	
TR2	.1E-8	
PØ	.1	
TR4	Ø.	(memory cell feedback)
TR3	Ø.	(antigen replication)
Q1	.1E6	
Q1M	Ø.	
Q2	.1E6	
Q2M	Ø.	
D2Ø	Ø.	
D2ØØ	Ø.	

Note1: These constants were used for all calculations.

For memory cell feedback, TR4 was changed to .1

For antigen replication, TR3 was changed to .1E-19

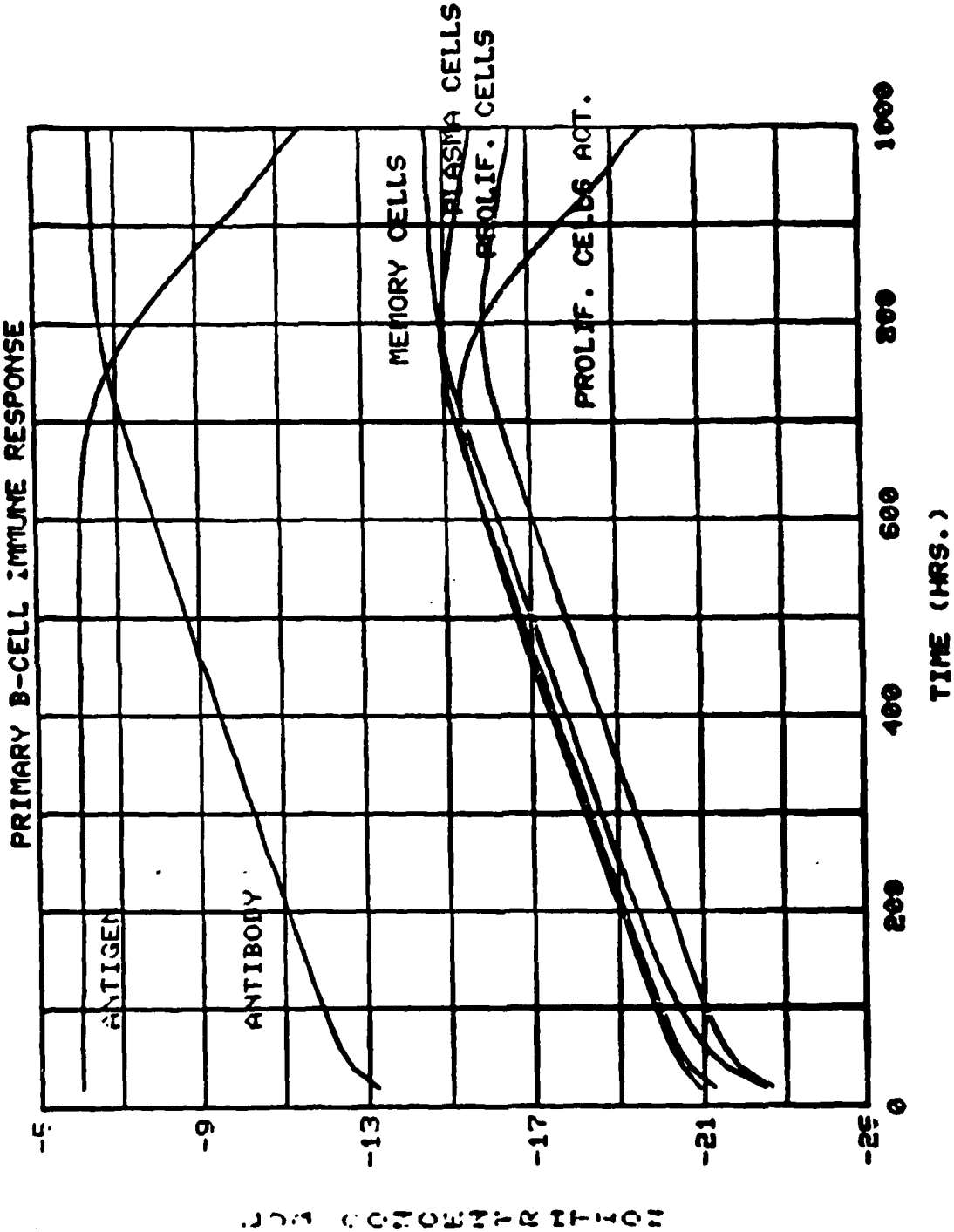
C. Initial Conditions

Identity	Concentration (Moles/Liter)
Target Cell, Ø Sites	.1E-19
Target Cell, 1 Sites	Ø
Target Cell, 2 Sites	Ø
Target Cell, 3 Sites	Ø
Antigen	.1E-5
Proliferating Cell, Activated	Ø
Proliferating Cell, Non-Activated	Ø
Memory Cell	Ø
Plasma Cell	Ø
Antibody	Ø
Antigen Antibody Complex	Ø

Note:

1. For the secondary immune response with memory cell feedback, it is hypothesized that the population of target cells at the beginning of the secondary response is equal to the population of memory cells at the end of the primary response, therfore for the secondary immune response calculation, the initial target cell population was .1E-11.

Figure 11.


PRIMARY B-CELL IMMUNE RESPONSE
ANTIGEN
ANTIBODY
MEMORY CELLS
PLASMA CELLS
PROLIF. CELLS
PROLIF. CELLS ACT.
-5
-9
-13
-17
-21
-25
0
200
400
600
800
1000
TIME (HRS.)

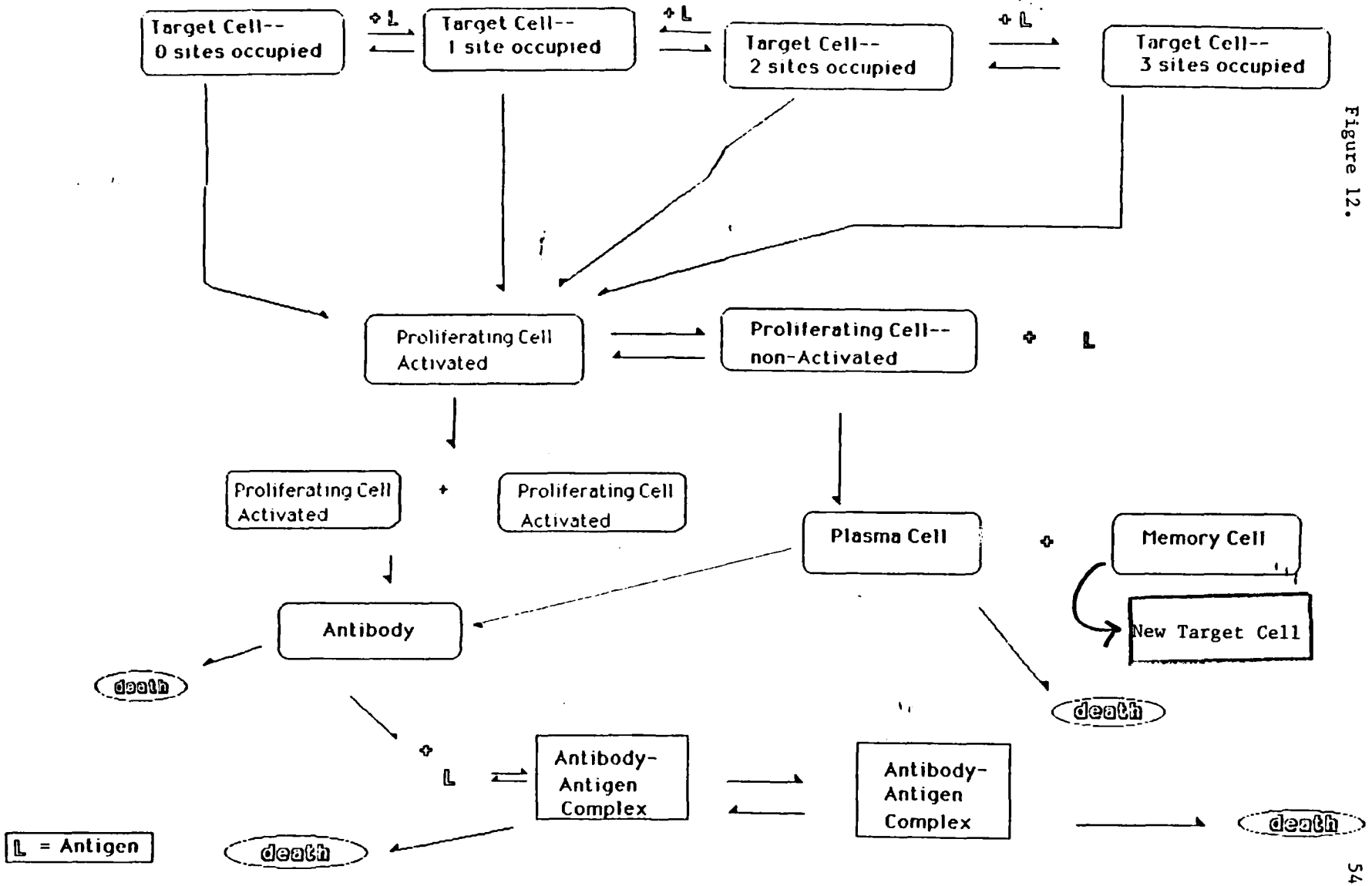
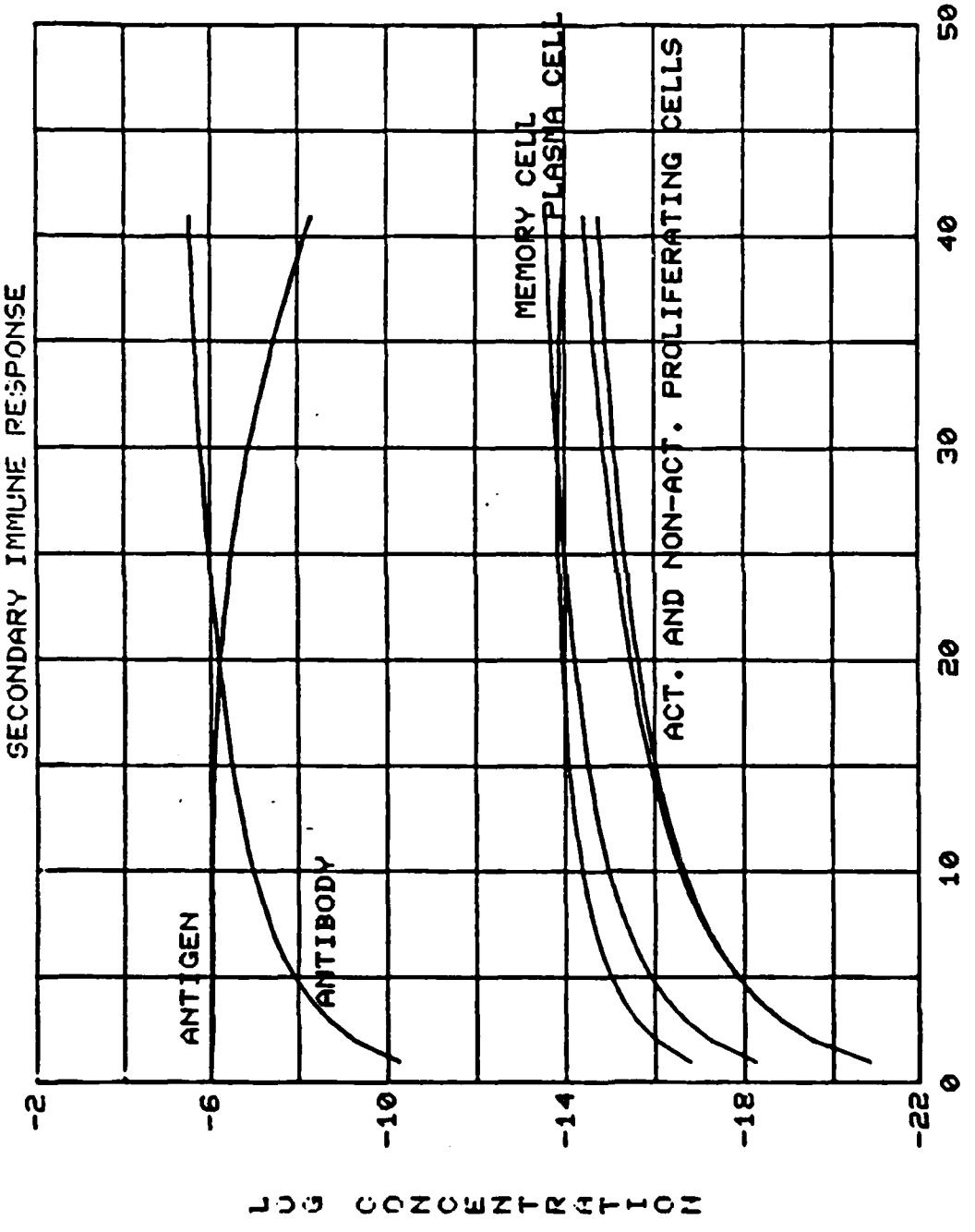


Figure 12.

Figure 12. Diagram of the T-cell independent B-type immune response with memory cell feedback. The rate constants are as indicated on figure 10.

Figure 13.


SECONDARY IMMUNE RESPONSE
ANTIGEN
ANTIBODY
MEMORY CELL
PLASMA CELL
ACT. AND NON-ACT. PROLIFERATING CELLS
LOG CONCENTRATION
TIME (HRS.)
-2
-6
-10
-14
-18
-22
0
10
20
30
40
50

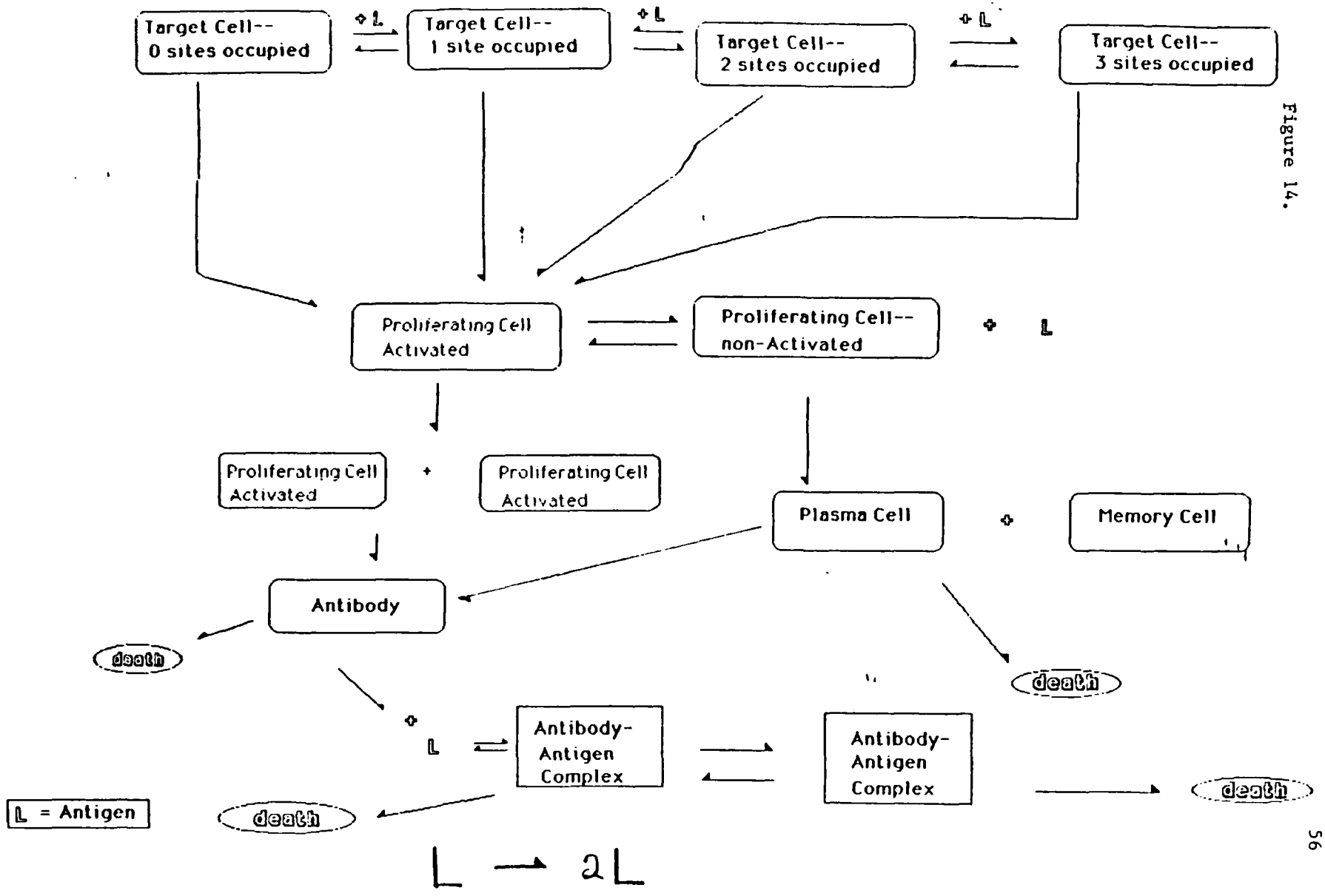
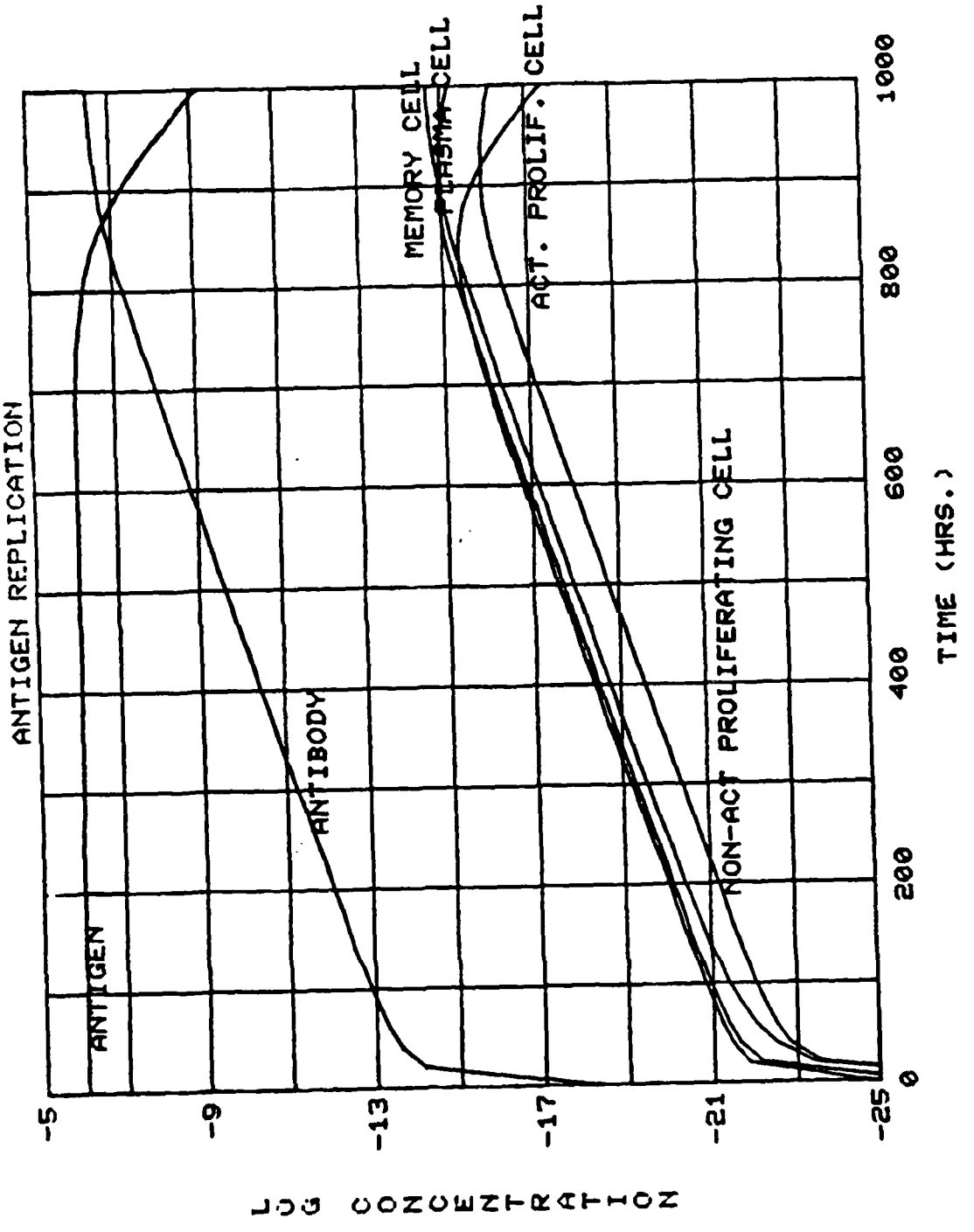


Figure 14.

Figure 14. Diagram of the T-cell independent B-type immune response with antigen replication.

Figure 15.


ANTIGEN REPLICATION
ANTIGEN
ANTIBODY
MEMORY CELL
PLASMA CELL
ACT. PROLIF. CELL
NON-ACT PROLIFERATING CELL
-5
-9
-13
-17
-21
-25
0
200
400
600
800
1000
TIME (HRS.)
LOG CONCENTRATION

Footnotes

[1] J. Higgins, "A Chemical Mechanism for Oscillation of Glycolytic Intermediates in Yeast Cells," Proc. Natl. Acad. Sci. USA., 51 (1964), pp 989-994.

[2] R.M. May, "Simple Mathematical Models with very Complicated Dynamics," Nature, 261 (1976), pp 459-467.

[3] A. Goldbeter, B. Hess, and R. Lefever, "Temporal, Spatial, and Functional Order in Regulated Biochemical and Cellular Systems," Adv. Chem. Phys. (1978), pp 393-406.

[4] G.I. Bell, "Mathematical Model of Clonal Selection and Antibody Production," J. Theor. Biol. 29 (1970), pp 191-232.

[5] G.I. Bell, "Mathematical Model of Clonal Selection and Antibody Production II," J. Theor. Biol. 33 (1971), pp 339-378.

[6] G.I. Bell, "Mathematical Model of Clonal Selection and Antibody Production III. The Cellular Basis of Immunological Paralysis," J. Theor. Biol. 33 (1971) pp 379-398.

[7] R.N. Hyer, Original Trident Project Proposal for Trident Scholars program, "A Computer-Designed Model of Enzyme Systems," March 2, 1984.

[8] H.M. Dintzis, R.Z. Dintzis, and B. Vogelstein, "Specific Cellular Stimulation in the Primary Immune Response: A Quantized Model," Proc. Natl. Acad. Sci USA., 79 (1982), pp 395-399.

[9] A.S. Perelson, "Paradoxes in B Cell Stimulation by Polymeric Antigen and the Immunon Concept," submitted to Paradoxes in Immunology.

[10] K. Arms and P.S. Camp, Biology, 2nd ed. (New York: CBS College Publishing, 1982), pp 540-541.

[11] Arms, pp 541-542.

[12] L.E. Hood, I.L. Weissman, J.H. Wilson, and W.B. Wood, Immunology (Menlo Park, Calif.: The Benjamin/Cummings Publishing Company, Inc.), pp 2-4.

[13] Arms, p. 541.

[14] Hood, pp 2-3.

[15] H.M. Dintzis, R.Z. Dintzis, and M.H. Middleton, "Studies on the Immunogenicity and Tolerogenicity of T-Independent Antigens," The Journal of Immunology, 131 (1983), pp 2196-2203.

[16] W.E. Paul, Fundamental Immunology (New York: Raven Press, 1984), p. 10.

[17] B.D. Davis, R. Dulbecco, H.N. Eisen, and H.S. Ginsberg, Microbiology, 2nd ed. (New York: Harper and Row, 1973), p. 352.

[18] G.I. Bell and A.S. Perelson, Theoretical Immunology (New York: Marcel Dekker, 1978), p. 4.

[19] P.A. Small, P.M. Small, and P.A. Small, Jr., "Understanding Immunology", pamphlet from Carolina Biological Supply Company, Burlington, N.C., pp 4-5.

[20] Bell and Perelson, p. 6.

[21] Bell and Perelson, pp 6-7.

[22] F.M. Burnet, Immunology (San Francisco: W.H. Freeman, 1976), pp 12-21.

[23] G.I. Bell and A.S. Perelson, Theoretical Immunology, pp 4-14.

[24] Hood, pp 1-2.

[25] Hood, pp 17-25.

[26] Hood, p. 11.

[27] B.A. Waite, unpublished work on a theoretical model of the lifetime of bound hapten clusters, May 1985.

[28] Davis, p. 357.

[29] C. DeLisi and A.S.Perelson,"Receptor Clustering on a Cell Surface. I. Theory of Receptor Cross-Linking by Ligands Bearing Two Chemically Identical Functional Groups," Mathematical Biosciences, 48 (1980), pp 71-110.

[30] C. DeLisi, "Theory of Clustering of Cell Surface Receptors by Ligands of Arbitrary Valence: Dependence of Dose Response Patterns on a Coarse Cluster Characteristic,"

Mathematical Biosciences, 52 (1980), pp 159-184.

[31] A.S. Perelson, "Receptor Clustering on Cell Surface. III. Theory of Receptor Cross-Linking by Multivalent Ligands: Description by Ligand States," Mathematical Biosciences, 53 (1981), pp 1-39.

Bibliography

Arms, Karen, and Pamela S. Camp, Biology, 2nd ed., New York: CBS College Publishing, 1982.

Bell, George I., "Mathematical Model of Clonal Selection and Antibody Production," J. Theor. Biol., 29 (1970), pp 191-232.

Bell, George I., "Mathematical Model of Clonal Selection and Antibody Production. II.," J. Theor. Biol., 33 (1971), pp 339-378.

Bell, George I., "Mathematical Model of Clonal Selection and Antibody Production III. The Cellular Basis of Immunological Paralysis," J. Theor. Biology, 33 (1971), pp 379-398.

Bell, George I., "Model for the Binding of Multivalent Antigen to Cells," Nature, 248 (1974), pp 430-431.

Bell, George I., "Predator-Prey Equations Simulating an Immune Response," Mathematical Biosciences, 16 (1973), pp 291-314.

Bell, George I. and Alan S. Perelson, Theoretical Immunology, New York: Marcel Dekker, 1978.

Burnet, F.M., Immunology, San Francisco: W.H. Freeman, 1976.

Davis, Bernard D., Renato Dulbecco, Herman N. Eisen, Harold S. Ginsberg, and W. Barry Wood, Microbiology, 2nd ed. New York: Harper and Row, 1973.

DeLisi, Charles, "Theory of Clustering of Cell Surface Receptors by Ligands of Arbitrary Valence: Dependence of Dose Response Patterns on a Coarse Cluster Characteristic," Mathematical Biosciences, 52 (1980), pp 159-184.

DeLisi, Charles and Alan S. Perelson, "Receptor Clustering on a Cell Surface. I. Theory of Receptor Cross-Linking by Ligands Bearing Two Chemically Identical Functional Groups," Mathematical Biosciences, 48 (1980), pp 71-110.

Dintzis, Howard D. and Renee Z. Dintzis, and Marjorie H. Middleton, "Studies on the Immunogenicity and Tolerogenicity of T-Independent Antigens," The Journal of Immunology, 131 (1983), pp 2196-2203.

Dintzis, Howard M., Renee Z. Dintzis, and Brian Vogelstein, "Specific Cellular Stimulation in the Primary Immune Response: A Quantized Model," Proc. Natl. Acad. Sci. USA, 79 (1982), pp 395-399.

Goldbeter, A., B. Hess, and R. Lefever, Temporal, Spatial, and Functional Order in Regulated Biochemical and Cellular Systems, Adv. Chem. Phys., pp 393-406.

Higgins, Joseph, "A Chemical Mechanism for Oscillation of Glycolytic Intermediates in Yeast Cells," Proc. Natl. Acad. Sci. USA, , 51 (1964), pp 989-994.

Hood, Leroy, Irving L. Weissman, John H. Wilson, and William B. Wood, Immunology, 2nd ed. Menlo Park, California: The Benjamin/Cummings Publishing Company, Inc., 1984.

May, Robert M, "Simple Mathematical Models with very Complicated Dynamics," Nature, 261 (1976), pp 459-467.

Paul, W.E., Fundamental Immunology, New York: Raven Press, 1984.

Perelson, Alan S., "Paradoxes in B Cell Stimulation by Polymeric Antigen and the Immunon Concept," submitted to Paradoxes in Immunology.

Perelson, Alan S, "Receptor Clustering on Cell Surface III. Theory of Receptor Cross-Linking by Multivalent Ligands: Description by Ligand States," Mathematical Biosciences, 53 (1981), pp 1-39.

Small, Parker A., Peter M. Small, and Parker A. Small, Jr., "Understanding Immunology," pamphlet from Carolina Biological Supply Company, Burlington, North Carolina, pp 4-5.

Waite, Boyd A., unpublished work on a theoretical model of the lifetime of bound hapten clusters, May 1985.

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