AN ABSTRACT OF THE THESIS OF

Zarreen H Farooqi for the degree of Doctor of Philosophy in Electrical & Computer Engineering presented on December 17,1986 .

Title: Identification of Stochastic Systems With Random Parameters With Particular Reference to the Recirculating Lymphocytes in the Immune System .

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Abstract Approved:

Ronald R. Mohler

This thesis is composed of four parts: i) system description, ii) model development, iii) parameter estimation, and iv) validation. The natural system used here is an aspect of the immune system, namely, the distribution of recirculating lymphocytes in various organs throughout the body. This distribution gains importance because: i) it is consequential in effective defense of the body, and ii) lymphocyte maldistribution may be a symptom of a disease state.

Certain deterministic models of lymphocyte distribution have been published previously. Here,

discrete-time and continuous-time stochastic models are developed. The class of models studied here are closed compartmental. The derived structures are a vector bilinear time series with two inputs (or random coefficient autoregression) and a vector stochastic differential equation, respectively, for the discrete and the continuous cases. Various properties of the solutions are studied. Parameter estimation for a 7-compartment system is done using nonlinear optimization with weighted least squares and -2 ln likelihood criteria (assuming Gaussianity for convenience, though not completely realistic). The outputs of the models are statistically examined against best available experimental data. The residual errors are analyzed for proximity of fit, validity of the models, Gaussianity, and stationarity. Multiple comparisons are performed to test lack of fit of individual compartments and in so doing major sources of error in estimation are assessed.

The particular class of models studied here are structurally unstable. The means are marginally stable and for the estimated values of the parameters the variances diverge.

IDENTIFICATION OF STOCHASTIC SYSTEMS WITH RANDOM PARAMETERS WITH PARTICULAR REFERENCE TO THE RECIRCULATING LYMPHOCYTES IN THE IMMUNE SYSTEM

by

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The experimental data was obtained from the Department of Pathology, University of Manchester, England. The sad demise of Prof. W.L. Ford in whose laboratory the data had been collected was a loss to his profession. Help provided by him during his lifetime is greatly appreciated by the author.

The main computer center of the University was used a little, but for the most part the HP-1000 in the Department of Electrical & Computer Engineering was used. The staff associated with these facilities, especially the latter, were always very helpful.

Finally, the author continues to accept in gratitude the support provided by his wife during the production of heaps of computer output, its interpretation, and the writing of the thesis.

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CHAPTER 1

INTRODUCTION:

MATHEMATICAL AND PHYSIOLOGICAL PRELIMINARIES

1.0 INTRODUCTORY REMARKS

Nature has always fired the curiosity of man leading to philosophical speculation, modeling at different levels (perceptual ,linguistic, physical, mathematical and philosophical), and consequent

understanding and integration with previous knowledge. It has been observed time and again that in diverse phenomena when appropriate assumptions are made, the structure of the mathematical model turns out to be the same. Enns et.al. (1981) note in their preface that

> " The nonlinear models involved [in nature] often span several different disciplines, a simple example being the Volterra-type model in population dynamics which has its analog in nonlinear optics and plasma physics (the 3-wave problem), in the discussion of social behavior of animals, and in biological competition and selection at molecular level."

This is the case with a variety of other structures. Thus results derived from or applicable to a mathematical model for a biological system can have potential implications for applications in other areas when given proper and relevant interpretation. This is the case of the present project.

After discussing the purpose of this research and the proposed plan of the thesis, this chapter will review compartmental analysis, the immune system, and its mathematical models.

1.0.1 RELEVANCE OF THIS RESEARCH.

Many biological phenomena have been modeled from different points of view. The immune system is one of the physiological systems that has been modeled at various hierarchical levels: subcellular, cellular, and organismic. This research concerns cell populations and thus links together many of the processes that occur at the cellular and subcellular levels, namely, i) cells providing defense, physically and biochemically, ii) cells conveying information by physically moving from one region of the body to another (e.g., antigen-macrophage interaction, T-B cell cooperation), and iii) information transmission through hormone-like activity of agents released by the cells (e.g., lymphokines and antibody). The group of processes treated here is collectively termed LYMPHOCYTE CIRCULATION or sometimes RECIRCULATION since most of the lymphocytes involved experience the phenomena repeatedly in their lifetime. The focus here is on the distribution (to be precise, on quantification of the 'norm' of the distribution) of the recirculating lymphocyte pool (RLP). This is important because i) the RLP is consequential in effective defense of the body, ii) a maldistribution can be a symptom of a disease state, and iii) different patterns of maldistribution

may be indicative of different diseases.

This project can be of interest to systems scientists, applied mathematicians, statisticians, physiologists, immunologists, and medical researchers. A knowledge of differential equations, bilinear time series (or random coefficient autoregression), stochastic differential equations, and aspects of numerical analysis is assumed. For mathematically oriented biologists, who might not be familiar with some of mathematical aspects, fairly detailed appendices are included. It is impossible, especially for a PhD dissertation, to be self-contained. Thus an attempt has also been made to compile a good, up-to-date, relatively comprehensive (though not exhaustive) collection of references.

1.0.2 PLAN OF THE THESIS.

This project is an attempt to collect various mathematical and physiological items together and combine them in a happy marriage. In order to describe and quantify the processes mentioned before the following plan will be adopted. To start with, some mathematical situations that will arise later

(compartmental analysis) are reviewed. Related propositions are given in appendices. To keep the biological aspects in perspective, a brief outline of the immune system and some models of its diverse functions are discussed (e.g., humoral immune response, cytotoxicity, disease). For the sake of brevity and lest the digression be too severe, sometimes only the sources are given. Then the processes associated with recirculation are focussed on and a few related experimental techniques discussed. The paper by Smith & (late) Ford, 1983, which is the source of all the experimental data that has been used in this study, is considered and the published deterministic, linear (time-invariant, time-variant, and time-delay) and nonlinear models presented. After discussing the need for stochasticity, the discrete-time and continuous-time stochastic models are developed, their parameters interpreted through hemodynamic arguments, and their theoretical structure investigated for stability, second-order stationarity, and strict stationarity. Parameter estimation is done using weighted least squares and -2 ln likelihood minimizing criteria. To estimate the deterministic part of the parameters, first-moment equations are used for both models. For noises (both multiplicative and additive) the mean is

assumed zero and the variances are estimated from the second-moment equations. To investigate the validity of the stochastic models detailed statistical analyses are performed on the residual differences between experimental and estimated states. In the end, the possible relations between the two stochastic models are examined, their lack of fit with the experimental data analyzed, the inadequacy of the estimation procedures and of the data discussed, suggestions for improvement made, and possible conclusions drawn.

1.1 MATHEMATICAL PRELIMINARIES.

Until recently, only arithmetic and classical statistics were the mathematical tools used by biologists. Of late, other areas of mathematics have started making inroads into life sciences. Historically, works of Vito Volterra, Lotka and Rashevsky pointed in that direction. The desire to mimic the "inherent purposiveness" and other properties of biological systems in construction of artificial systems has given additional impetus to analytical studies. Limitations of human thought combined with the number of variables and their interactions to be

considered simultaneously in nonlinear biological processes create difficulties which can only be surmounted by good experimental design and the use of available compatible analytical tools.

Compartmental analysis is one of the tools that has been used. It will be discussed here and some of the results are given in Appendix 2. There is a brief discussion of multiplicative processes in Appendix 1.

1.1.1 COMPARTMENTAL ANALYSIS.

Physiological systems can be treated as interacting subsystems , which, if further structure is ignored, can be renamed compartments and their interaction analyzed as such. Tracing the origins of compartmental analysis would mean working through the history of differential equations and would take us too far from the topic, but the present application arose in relation with pharmacokinetics (Teorell, 1937a; Teorell, 1937b) and with studies using radioactive tracers (Zilversmit, et.al., 1943). The term "compartment" was introduced by Sheppard (1948). Subsequently, many books appeared reviewing compartmental modeling: Sheppard (1962), Atkins (1969), and Jacquez (1972), the last

being a standard reference up to now. Since the 1950's ecosystem modeling using linear time-invariant system models has also contributed a great deal to compartmental analysis (O'Neill, 1979). Recent reviews of the subject include: Brown (1980), Godfrey (1983), Anderson (1983), and Lambrecht and Rescigno (1983), and there is also a critique by Zierler (1981). Most of the literature is in terms of linear time-invariant system analysis.

1.1.1.1 TYPES OF COMPARTMENTAL SYSTEMS.

When the compartments of a system are connected in series (or in a chain) and only adjacent compartments communicate, they form a CATENARY system. If there is one central or "mother" compartment and all others communicate with it as "daughter" compartments, they form a MAMILLARY system. Both are diagrammed in Figure 1.

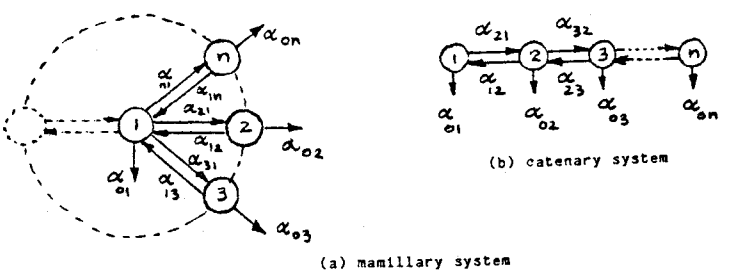


Figure 1. Types of compartmental systems. (Godfrey, 1983)

If material does not pass out of the system it is considered CLOSED , otherwise it is OPEN. A subsystem of compartments which receives input from the remainder of the system but does not transfer out of the subsystem (i.e. a closed subsystem) is called a TRAP.

1.1.1.2 ASSUMPTIONS.

It is implicit in the term compartmental analysis that it considers a lumped system with only a finite number of compartments. Besides, it is assumed that the compartments are homogeneous and well-mixed; particles of a particular species are indistinguishable from one another; all particles in a compartment have the same probability of transition, upon entrance into a compartment they mix instantly, and that the amount of tracer is very small compared to the tracee (so as not to influence the overall dynamics).

1.1.1.3 STRUCTURE OF COMPARTMENTAL SYSTEMS.

The general form of a compartmental system with n

compartments is

$$\dot{x}_i = f_{io} + \sum_{\substack{j=1 \\ j\neq i}}^{n} f_{ij} - \sum_{\substack{j=1 \\ j\neq i}}^{n} f_{ji} - f_{oi} \qquad \ldots(1.1)$$

$$i = 1,\ldots,n \ ,$$

where $f_{ij} = f_{ij}(t, \underline{\theta}, \underline{x})$ the function describing the flow rates from compartment j to compartment i; Subscript o denotes the environment.

$\underline{\theta} = \{a_{i1}, a_{i2}, \ldots, a_{in}\}$ the parameter set, a_{ij} being the system parameters.

$\underline{x} = [x_1, x_2, \ldots, x_n]'$, the state vector with components x_i.

t = time.

If $f_{ij} = 0$, $\forall$ i there is nothing excreted to the environment so that the system is closed; otherwise it is open. Physical considerations constrain the flow to be nonnegative ($f_{ij} \geqslant 0$, $\forall$ i,j). Thus compartmental models can also be considered as systems of first-order

constrained differential equations. Explicit expression of f_{ij} gives rise to various forms of systems discussed below.

1.1.1.4 LINEAR TIME-INVARIANT SYSTEMS (LTI).

When $f_{ij} = a_{ij} x_j$, a_{ij} being the rate constants, the system of equations becomes of the form

$$\dot{x}_i(t) = \sum_{\substack{j=1 \\ j\neq i}}^{n} a_{ij} x_j(t) - \sum_{\substack{j=0 \\ j\neq i}}^{n} a_{ji} x_i(t) + \sum_{j=1}^{m} b_{ij} u_j(t),$$

$$i = 1,\ldots,n , \qquad \ldots(1.2)$$

where $u_i(t)$ = input $(i = 1,\ldots,m)$.

Letting

$$a_{ii} = \left(+ \sum_{\substack{j=0 \\ j\neq i}}^{n} a_{ij}\right) , \quad i = 1,\ldots,n , \quad \ldots(1.3)$$

(1.2) becomes

$$\dot{x}_i(t) = \sum_{\substack{j=1 \\ j\neq i}}^{n} a_{ij} x_j(t) - a_{ii} x_i(t) + \sum_{j=1}^{m} b_{ij} u_j(t) . \qquad \ldots(1.4)$$

In vector form (1.4) is

$$\dot{\underline{x}}(t) = A\underline{x}(t) + B\underline{u}(t) , \qquad \ldots(1.5)$$

where $A = [a_{ij}]$, $B = [b_{ij}]$.

The n n system matrix A has the following properties:

i) $a_{ij} \geqslant 0$, $i \neq j$,

ii) $a_{ii} \leq 0$, and

iii) $\sum_{i=1}^{n} a_{ij} = -a_{oj}$, j.

Such matrices are called COMPARTMENTAL MATRICES and are column diagonally dominant since

$$|a_{ii}| \geqslant \sum_{\substack{j=1 \\ j \neq i}}^{n} |a_{ji}| \qquad i = 1,\ldots,n.$$

Physically this reflects conservation of matter or energy.

Similar matrices occur in studies of reactor criticality (Birkhoff & Varga, 1958) and their negatives in the theory of dielectric relaxation (Axilrod, 1956). They are related to Metzler matrices of economics

(Newman, 1959).

A is an essentially nonnegative matrix and many results are available for such matrices. See Appendix 1. Some results related to nonnegativity, boundedness, connectivity, stability, and identifiability of functions of A are given in Appendix 2.

Directed graphs have been used to study LTI compartmental systems. When nodes represent the compartments and the directed edges the transfer paths, the graph is referred to as a CONNECTIVITY DIAGRAM. If starting from any compartment, material can pass to any other compartment in the system by some path, then the system and its associated connectivity diagram (and its compartmental matrix) is called STRONGLY CONNECTED.

1.1.1.5 LINEAR TIME-VARYING SYSTEMS (LTV).

If the rate coefficients a_{ij} are treated as functions of time $a_{ij}(t)$ then $a_{ij} = a_{ij}(t)$, and the system is called linear time- varying.

$$\dot{x}_i(t) = \sum_{\substack{j=1 \\ j \neq i}}^{n} (a_{ij}(t)x_j(t) - a_{ji}(t)x_i(t))$$

$$- a_{oi}(t)x_i(t) + \sum_{j=1}^{m} u_j(t)$$

or ...(1.6)

$$\dot{\underline{x}}(t) = A(t)\underline{x}(t) + \underline{u}(t)$$

Reasoning from conservation of matter Mazanov (1976) demonstrated the stability of the solution of (1.6), thinking of A(t) as a time-varying compartmental matrix (see Appendix 3, propsition A3.1).

Many physiological functions exhibit periodicity (circadian or other) so that periodically varying rate coefficients with period T are important

$$a_{ij}(t) = a_{ij}(t+T) \qquad ...(1.7)$$

There is periodicity in ecosystems also, reflecting variations in light, temperature, and seasons.

Although periodicity will not be encountered in this project, it is of interest to note that Mulholland & Keener (1974) have shown that, if initially all the compartments in a time-varying system contain some material then some material remains in them thereafter, i.e. the amount never becomes a negative quantity. If the system is periodic and has a positive, periodic input with the same period then it has a unique periodic

solution. In the case of an open system with no inputs, there is no periodic solution and each state decays asymptotically to zero (see Prop A3.2)

Another form of variation occurs when some or all of the rate coefficients change suddenly from one value to another. Such changes may be due to exercise in a subject who was previously resting or due to food intake when fasting.

1.1.1.6 NONLINEAR SYSTEMS (NL).

In various studies the flows, f_{ij} , have been related to the states in different ways. If

$$f_{ij} = a_{ij} f_j(x_j) \qquad i = 0,1,\ldots,n; \qquad j = 1,\ldots,n \ ; \ i \neq j \ , \qquad \ldots(1.8)$$

where $a_{ij} \geqslant 0$ and constant, $f_j(x_j) > 0$.
Thus (1.1) becomes

$$\dot{x}_i(t) = f_{io} + \sum_{\substack{j=1 \\ j \neq i}}^{n} [a_{ij} f_j(x_j) - a_{ji} f_i(x_i)] - a_{oi} f_i(x_i) \ . \qquad \ldots(1.9)$$

In some cases, especially in pharmacokinetics and metabolic systems, the flows have been modeled as capacity-limited (i.e. following Michaelis-Menten kinetics):

$$f_{ij} = \frac{V_m}{K_m + x_j(t)} x_j(t) , \qquad ...(1.10)$$

where V_m and K_m are constants.

In other cases Langmuir saturation (Kruger-Thiemer et.al, 1964) has been used

$$f_{ij} = a_{ij} (1 - \sigma_i x_i(t)) x_j(t) , \qquad ...(1.11)$$

where σ_i is a constant such that $(1 - \sigma_i x_i(t)) \geqslant 0$.

Michaelis-Menten kinetics has considerable importance as it can be used whenever there is a capacity-limited elimination of substances e.g. enzyme kinetics, metabolism, renal excretion, liver function. Compartmental systems with such kinetics are discussed in Tong & Metzler (1980) and many examples are given in Gibaldi & Perrier (1982) and Carson, Cobelli, and Finkelstein (1983).

A more general case compared to (1.8) is when

$$f_{ij} = a_{ij}(\underline{x})\, x_j \qquad \ldots(1.12)$$
$$i = 0,1,\ldots n;\ j = 1,\ldots,n;\ i \neq j.$$

so that the nonlinear representation of (1.6) may be written as

$$\dot{\underline{x}}(t) = f(\underline{x}) + \underline{u}\ . \qquad \ldots(1.13)$$

It has been shown by Maeda, Kodama, & Ohta (1978) that with certain constraints on $a_{ij}(.)$ in (1.12), nonnegativity of the off-diagonal elements in the nonlinear compartmental matrix implies nonnegativity of the state of the system. With additional conditions on $a_{ij}(.)$ of these off-diagonal elements, stability and uniqueness of the equilibrium set is ensured.

Lewis & Anderson (1980) have shown that if arbitrary time-delays (including varying ones) are introduced in intercompartmental transfers, system properties like nonnegativity, boundedness, and stability are not affected. Ladde (1976) has also analyzed the stability of open systems of the form

$$\dot{\underline{x}}(t) = A(t,\underline{x})\ \underline{x}(t) \qquad \ldots(1.14)$$

1.1.1.7 STOCHASTIC COMPARTMENTAL SYSTEMS.

Stochastic variability in physiological experiments can have many and varied causes - for example, changes in cell volume/area ratio, changes in membrane permeability, presence of drugs other than the one administered, changing environmental and measurement conditions, or presence of only a relatively small number of particles of interest within each compartment.

Variablity can be incorporated either by making the transfer rate coefficients stochastic, or by keeping them constant and thinking of the state as random variable (or vector). In both cases the state ends up as a stochastic process. Additive noise may also drive the state to take into account some of the sources of variability that might otherwise have been neglected by the model.

1.1.1.7.1 STOCHASTIC VARIABILITY OF RESPONSES.

Matis & Wehrly (1979) review this approach. Briefly, if there are N subjects, each with n compartments, there will be N responses (to a tracer, say) and curve-fitting each response gives a sum of

exponentials

$$x_j(t) = \sum_{i=1}^{n} A_i \exp(\lambda_i t), \quad t \geqslant 0$$
$$j = 1,\ldots,N$$

The N values of A_i and λ_i $(i = 1,\ldots,n)$ may be treated as sample values of random variables, and if N is large, the sample statistics can be regarded as a reasonable approximation of the population. It is generally assumed that $\{A_i\}$ and $\{\lambda_i\}$ are independent and identically distributed (i.i.d.), stationary sequences, and that the sequences are independent of one another. The distribution of $-\lambda_i$ should be nonnegative. Thus, in a sense, estimation of stochastic parameters becomes a deterministic problem. The minimizing criterion used may be a weighted sum of squares of differences between mean values of observed x(t) at each observation time point and the mean values estimated from the model.

1.1.1.7.2 STOCHASTIC RATE COEFFICIENTS.

Instead of taking the rate coefficients static as above, they can be considered as varying dynamically. Comparatively little literature is available in this

case. Jacquez (1972) mentioned it and Cobelli & Morato (1978) have presented an estimation procedure using nonlinear filters.

1.1.1.7.3 STOCHASTIC STATE VARIABLES.

Here the rate coefficients are assumed either constant or time-varying deterministic and the state variables as stochastic processes. Such models are of particular interest when the number of particles in a compartment are small, they are assumed to move independently of one another and their transitions are thought to be Markovian. Purdue (1979) has reviewed this approach.

1.1.1.7.4 MISCELLANEOUS.

There have been attempts at making more general stochastic models. Matis & Tolley (1979) combined a number of possible sources of stochasticity to give a general framework for stochastic modeling. Some researchers have generalized the Markov interaction process to a Semi-Markov process (Weiner & Purdue, 1977;

Marcus, 1979). Combined use of additive and parameteric noises does not seem to have received much attention in compartmental models.

1.2 THE IMMUNE SYSTEM.

All animals have defense mechanisms to protect them from invasion of foreign bodies (e.g. viruses, microbes, etc.). In mammals there are several lines of defense, the first of which is the passive, physical protection of the skin. For agents that somehow penetrate the first line of defense and are recognized as potentially harmful (i.e. as non-self) there are several other mechanisms. These include those that are non-specific (or innate) and those that are specific (or acquired) (Guyton, 1976). Both of these types of immunity may be either humoral or cellular. There is a continuous interaction between all these different mechanisms. This project will be concerned only with acquired immunity, the existence of all the other defense mechanisms and their interactions being assumed. These may to a degree contribute to the "noise" due to uncertainty in the models to be derived later. For a brief review of the immune system see Appendix 8. Most

of the relevant vocabulary is covered there and in the Glossary (Appendix 10).

1.2.1 LYMPHOCYTE MIGRATION.

There is substantial evidence of migration of lymphocytes (See section 2.1). Opinions as to the functions of this migration or circulation in blood and lymph vary (See section 2.1.6). Yet it seems that this circulation and recirculation may be an effective way of ensuring that the relevant specific lymphocytes interact with antigen and with each other, and that memory cells (i.e. primed lymphocytes) become disseminated to the various lymphoid tissues in the body (Greaves, Owen, & Raff, 1974). Thus lymphocyte migration could be thought of as consequential in control of infection and disease.

Lymphocytes basically exhibit two traffic patterns: i) homing, and ii) recirculation. Homing refers to cell migration from one site to another, while in recirculation there is continuous movement between, and through, the lymphoid tissues (McConnell, Munro, & Waldman, 1981). There are cells which may be thought of as "organ-seeking", i.e. they home-in on a particular organ and remain there.

Recirculation was first conjectured by Sjoval (1936) and demonstrated by Gowans and his colleagues (Gowans, 1959; Gowans & Knight, 1964). Figure 2 demonstrates the different traffic patterns.

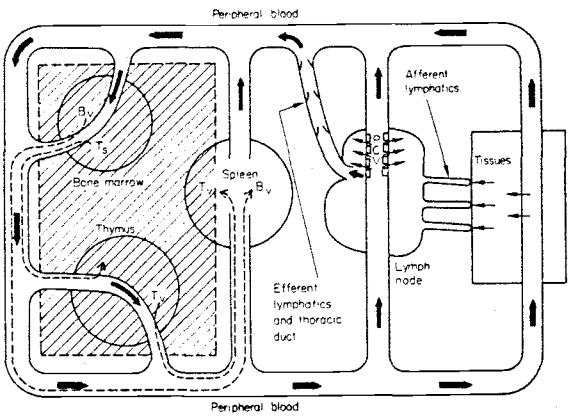


Figure 2. Major lymphocyte traffic patterns in an adult. Key: □ represent recirculating pool (RLP); ▨ not part of RLP. (McConnell, et.al., 1981)

The unhatched areas in the diagram form the recirculating lymphocyte pool (RLP) while the hatched area refers to the homing pattern of lymphocyte traffic. High endothelial venules (HEV) are the sites of lymphocyte migration into lymph nodes and elsewhere.

1.2.2 FORMATION OF THE RLP.

Factors that control entry of cells into the RLP are poorly understood. There are two possibilities: i)

cells continually leave the primary lymphoid organs and directly enter the RLP as potentially long-lived cells, and ii) cells leaving the primary tissue undergo a phase of "maturation" in the secondary lymphoid tissue (e.g. contact with antigen) before entering the RLP (Sprent, 1977). There is more evidence for the latter and as such the formation of the RLP is illustrated in figure 3 which is a combination of figures 2 and 13.

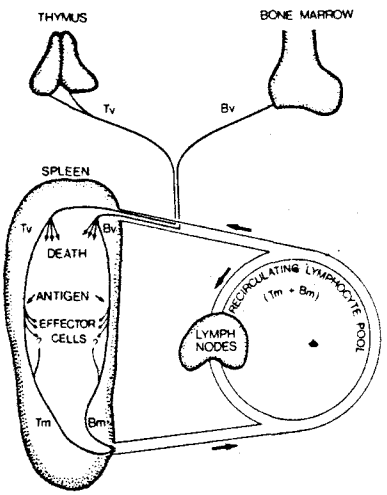


Figure 3. A schematic of the formation of RLP. Key: v = virgin; m = memory. (Sprent, 1977)

It is assumed that the virgin cells are short-lived. If they do not come into contact with an antigen they die soon, otherwise they mount a primary response to the antigen. This encounter determines that some of the responding cells are triggered to form long-lived

recirculating memory (B_m,T_m) cells. According to this notion, most, perhaps all, recirculating cells are memory cells, and any response they participate in is a secondary response. More on recirculating lymphocytes will be presented in Chapter 2. Their models will also be discussed there.

1.3 MATHEMATICAL MODELING IN IMMUNOLOGY AND BIOMEDICINE.

As mentioned earlier, of late there has been considerable activity in mathematical modeling of complex natural phenomena. Numerous conferences and many recent books attest to this: the Springer-Verlag Lecture Notes in Biomathematics, Rubinow (1975), Bell, Perelson, & Pimbley (1978), Fedina et.al. (1981), Carson, Cobelli, & Finkelstein (1983), Marchuk (1979b), Marchuk (1983), Marchuk & Belykh (1983), Moller, Popovic, & Thiele (1983). In some cases biological models have been included in conferences in physical sciences (Arnold & Lefever, 1981; Avula et.al., 1984). There have been biomedically related papers in many IFIP Working Conferences also. There has also been an interest in building stochastic models, two examples being the Trento (Italy) conference from which the

papers were published under the title "Stochastic Methods in Life Sciences: General Aspects and Specific Models" as a special issue of the Bulletin of Mathematical Biology (DeLisi, Iannelli, & Koch, 1983) and the special issue of the Acta Applicandae Mathematica (Koch & Hazewinkel, 1985) on "Mathematics of Biology". Several reviews have appeared on the models of the immune system: Merrill (1980), Mohler, Bruni, & Gandolfi (1980) and DeLisi (1983). Many models have been proposed related to certain aspects of the immune system, in particular the immune response. Some aspects of such modeling are reviewed in Appendix 9.

CHAPTER 2

RECIRCULATING LYMPHOCYTES AND MATHEMATICS OF THEIR DISTRIBUTION

2.0 INTRODUCTORY REMARKS.

An overview of the immune system and models of some of its aspects were discussed in the previous chapter. Apparently the discrete nature of the immune system with strategically located organs requires efficient communication to integrate its functioning. Of the three major communication systems (nervous, endocrine, and circulatory) that can influence immune functions we will be interested only in the circulatory system in the present study. It is involved in information transmission, in transport of cells along their migratory paths, in removal of unwanted, dead and toxic matter from the body to appropriate sites of

excretion, and in the defense of the organism. The lymphatic system is a part of the circulatory system and functions to transport excess tissue fluid to the blood-stream and also to help defend the body against invasion by pathogens.

This chapter reviews the physiology of the recirculating lymphocytes, studies dealing with their distribution in the body, and the deterministic mathematical models thereof. Perchance quantification of the norm of distribution pattern and statistical analysis of deviation from the norm and/or maldistribution may be helpful in the diagnosis and estimation of disease severity, and in the classification and differential diagnosis of ecotaxopathies. Ecotaxopathy refers to abnormalities in migratory patterns, and some of its manifestations in man are: i) Hodgkin's disease, ii) Crohn's disease (gastrointestinal tract disease), iii) chronic liver disease (alcohol-related, hepatitis B, primary biliary cirrhosis), iv) skin diseases (psoriasis), v) connective tissue diseases (rheumatiod arthritis), vi) central nervous system diseases (multiple sclerosis), vii) solid tumors, viii) chronic lymphocytic leukemia, and many others.

2.1 RECIRCULATING LYMPHOCYTES & EXPERIMENTATION.

The existence of recirculating lymphocytes was hypothesized and demonstrated by Gowans (1959). (Also see sections 1.2.1 and 1.2.2) Subsequent work (Gowans & Knight, 1964; Ford & Gowans, 1969; Ford, 1969; Ford, 1975; Hall, Scollay, & Smith, 1976; DeSousa, 1981) has elaborated on the anatomy and physiology of recirculation.

A summary of the more important facts is presented below.

2.1.1 PROCESSES OF RECIRCULATION

There are three basic processes (Ford, Smith, & Andrews, 1978):

i) Lymphocytes leave the bloodstream by adhering to the surface of the vascular endothelium and subsequently crossing the capillary walls. Factors contributing to the mechanism of selection of cells that cross over are: a) hemodynamic and b) physico-chemical interaction between blood cells and vascular endothelium.

ii) Within lymphoid tissue, T-cells and B-cells

segregate from each other after they cross the capillary wall and reach the extravascular compartment.

iii) Each cell type returns to the blood by migrating along pre-defined paths within the tissues.

2.1.2 TRAFFIC IN LYMPHOID TISSUE.

Average lifespan in mouse of B-cells in thoracic-duct lymphocytes (TDL) is 5-7 weeks and of TDL-T cells 4-6 months (Ford,1975; DeSousa,1981). Different figures are reported in Elves (1972) and McConnell et.al. (1981). There is also a difference in the tempo of recirculation of T- and B- cells, the latter are much slower (Ford, 1975; DeSousa, 1981). TDL are mostly T-cells, only 15-20 % in mice and 20-35 % in rats being B-cells (Ford, 1975). T-cells recirculate in about 18 hours, of which less than one hour is spent in blood or lymph (Parrott & Wilkinson, 1981). Distribution of injected labeled lymphocytes in rat and plaice is shown in figure 4. Several factors influence the circulation of lymphocytes. Details may be obtained in Sprent (1977) and DeSousa (1981).

1. Antigenic factors

i) Non-specific effects (i.e. those that effect the whole RLP, not just specific cells) --- e.g., "trapping" and increase in blood flow to the lymph node(s) draining the infected area.

ii) Specific recruitment of cells. For example, effect on specific cells during trapping.

iii) Role in initiation, propagation, and persistence of immunity:

Initiation --- Both circulating and noncirculating lymphocytes participate in the development of the primary response. There is a possibility that a specific antigen may induce differentiation of noncirculating virgin T- or B-cells to recirculating cells (as seen in section 1.2.2)

Propagation --- Information about the arrival of an antigen is disseminated throughout the body by the RLP.

Persistence --- Most evidence suggests that recirculating lymphocytes are memory cells (See section 1.2.2).

2. Non-antigenic factors.

i) Phenotype of cell --- different cells have different microenvironmental preferences. Cell surface receptors, surface charge, size and cell

density affect circulation and movement through small vessels.

ii) Metal cations --- binding to Zn^{++}, Fe^{+++} can cause maldistribution.

iii) Agents that modify cell locomotion, e.g. Sodium azide interferes with selective interaction of cells with postcapillary endothelium.

iv) Irradiation --- B-cells are more radiosensitive than T-cells. In humans T-suppressor cells are more radiosensitive than T-helper cells.

v) Cortisone (or ACTH), Stress --- cause decreased TDL output. Continuous high levels of ACTH may cause lymphocyte lysis and inhibition of blood to lymph recirculation.

vi) Certain diseases may cause lymphocytopenia (e.g., Newcastle disease virus) or lymphocytosis (e.g., Bordetella pertusis).

vii) Anesthesia with ether leads to lymphocytopenia.

viii) Chemotactic and cellular factors some of which are mentioned in figure 11 (Appendix 8). Others include complement (C3 unit) and lymphokines. Lymphokines are active soluble factors (other than Ig's) released by lymphocytes, e.g., Migration inhibition factor (MIF), Leukocyte

migration inhibition factor (LIF), Macrophage chemotactic factor (MCF), Lymphocyte chemotactic factor (LCF), Lymphocyte trapping factor.

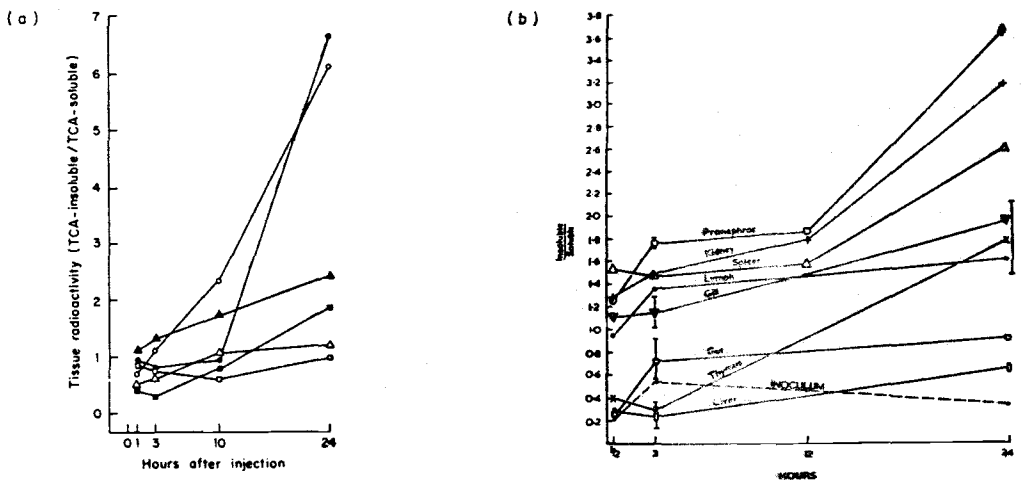


Figure 4. Recirculation of lymphocytes in rat and plaice (DeSousa, 1981)
(a) Radioactivity in samples of lungs (▲——▲), spleen (o——o), MLN (●——●), thymus (△——△), liver (□——□), and intestine (■——■) from rats injected intravenously with [^{3}H]-uridine-labelled TDL.
(b) Pattern of change in plaice with label injected into renal portal vein.

2.1.3 TRAFFIC IN NON-LYMPHOID TISSUE (Primarily based on DeSousa, 1981).

In most normal non-lymphoid tissue there are very few lymphocytes. The first response of skin to application of sensitizing agents or intradermal injections of antigen is usually the appearance of polymorphs or macrophages, although small or blast-like lymphocytes can be induced to extravasate 20-30 times above the baseline level (DeSousa, 1981). Mucosal sites, especially gut mucosa, normally contain lymphoid cells which differ in number and type from other non-lymphoid sites, e.g. skin. In non-mucosal sites the proportion of B-cells which extravasate is much smaller than T-cells. The latter predominate in human peripheral lymph and other extravascular fluids. Afferent or peripheral lymph contains very few lymphocytes, or considerable number of macrophages, and a different ratio of T:B cells (7.6:1) as compared to efferent lymph (2.5:1) and blood (2:1). After antigenic stimulation there is increased vascular permeability (DeSousa, 1981). Experiments suggest that use of a contact sensitizer can change blood flow and cell traffic. There is an increase in blood flow with a concomitant increase in lymphoblast migration and a small increase in small lymphocyte migration. There is

also a simultaneous increase in blood flow to the draining LN, but this is directly connected to small lymphocyte traffic and not to blast cell traffic (Ottaway & Parrott, 1979).

Gut associated (GALT) and bronchial associated lymphoid tissues (BALT) are continuously exposed to antigens and are thus more important of the mucosal sites. In human gut there are 10^5-10^6 cells/mm^3. The major classes of immunoglobulins (antibodies), IgA, IgM, and IgG, appear in the following approximate ratio 40:3:1. Small quantities of IgD and IgE are also present. During biopsies lymphocytes found in the human colon are larger than circulating lymphocytes. Of the mononuclear cells 58% are T-cells, 32% B-cells, and the remaining 10% monocytes or macrophages (DeSousa, 1981). In BALT there is a higher proportion of monocytes/macrophages compared to GALT: 50-70% of mononuclear cells are monocytes or macrophages and only 30-50% lymphocytes; of these, 68% are T-cells and 20.5% B-cells. Most cells in mucosal surfaces are effector cells rather than affector (or memory) cells. For more details see DeSousa (1981). Also, there was recently a conference on Mucosal Immune System (McGhee & Mestecky, 1983) whose proceedings contains a great deal of information.

2.1.4 CONTRIBUTIONS OF RLP TO THE PRIMARY AND SECONDARY RESPONSES.

1. The following events happen during the primary response (Ford, 1975):

i) Recruitment of migratory cells,

ii) Selective depletion of RLP,

iii) Selective enrichment of antigenically stimulated tissue,

iv) Specific recruitment of antigen-sensitive cells within the intravascular compartment.

2. The following happen during the secondary response (Ford,1975):

i) Dissemination of immune memory, (See section 2.1.2(1.iii))

ii) Specific recruitment of memory cells.

Antigen-sensitive T-cells are recruited from the RLP in both the primary and secondary responses (Ford, 1975; DeSousa, 1981). B-cells also are recruited in the secondary antibody response, but in the primary response most virgin B-cells reside in lymphoid tissue.

2.1.5 FACTORS PROMOTING EXTRAVASATION/MIGRATION (Parrott & Wilkinson, 1981).

Besides the factors mentioned in section 2.1.2 as influencing traffic in the lymphoid tissue, the following factors affect migration of cells, either directly or indirectly, throughout the body of the organism:

1. Blood flow,
2. Antigen-directed locomotion,
3. Cell-mediated immunity
4. Inflammation,
5. Temperature, and
6. Vascular permeability.

As seen in sections 2.1.1 and 2.1.3 blood flow is a very important factor in the migration of lymphocytes. In general, the flow velocity is proportional to the reciprocal of the effective cross-sectional area of the vessel. Different blood cells exhibit different flow properties. Most red blood cells tend to flow near vessel axis, while most lymphocytes and monocytes tend to flow near vessel walls (DeSousa, 1976).

Several factors are carried by the blood which are influential in modifying lymphocyte motion, e.g., antigen and chemoattractants. Chemokinetic factors,

lymphokines, and the presence of other cells (e.g. macrophages) can induce extravasation from capillary beds. Serum albumin acts as a chemokinetic agent and can influence lymphoblast motion once they are within the tissues (Also see Ottaway & Parrott, 1979). Lymphokines, are biologically active, soluble factors (other than antibodies) elaborated by stimulated lymphocytes. Some of these were mentioned in section 2.1.2(2.viii). There are several others (for details see Cohen, Pick, & Oppenheim, 1979, or any other book on lymphokines). Many lymphokines have a specific action, either helper, or suppressor, or an action on macrophages (e.g., Ag-dependent MIF). Other lymphokines have non-specific action: helper, supressor (MIFIF), action on inflammatory cells (MIF, LIF), action on vascular endothelium or other cells (TMIF), growth stimulation (LAF), and direct action on antigen (LT). Inflammation and cell-mediated immunity influence migration/extravasation of lymphocytes indirectly through the above factors .

Raised temperature of the body (or in the area of inflammation) creates an environment for increased migration of antigen and of lymphocytes (Marchuk, 1983).

With antigenic stimulation there is an increase

in vascular permeability and this results in leakage of protein from the vessels. It has been demonstrated experimentally that lymphoblast extravasation is not dependent on permeability (Rose & Parrott, 1977). See the note on serum albumin above.

2.1.6 FUNCTIONS OF LYMPHOCYTE MIGRATION / RECIRCULATION

(See section 1.2.1).

There has been speculation on what the functions of lymphocyte migration could be. Different suggestions include: i) information dissemination, ii) access of adaptive immunity to non-lymphoid tissue, and iii) some sort of physiological surveillance. All of these may have elements of truth in them.

Migration continually redistributes Ag-sensitive (i.e. memory) cells between the anatomically dispersed components of the peripheral lymphoid system thus affecting information dissemination and integrating the functioning of the immune system (Lozovoy & Shergin, 1979; Sprent,1977). It allows access of sensitized or effector lymphocytes to regions of antigen in non-lymphoid tissue e.g. skin.

Some researchers argue that recirculation is a physiological property of immunologically virgin lymphocytes as observed in a fetus, and as such must be a part of some sort of physiological surveillance. One view is that it could be involved in recognition and binding of metal ions as a protective device against metal toxicity and preferential use of indispensible metals, like Fe^{+++} and Zn^{++}, by bacteria or transformed cells (DeSousa, 1981).

2.1.7 METHODS USED TO STUDY MIGRATION.

The techniques to prepare and label lymphocytes are given in detail in Ford & Hunt (1973). To study cell traffic some sort of a marker is needed so that relevant cells can be tracked. Many different markers have been used:

1. Chromosomal markers --- T6, Barr bodies (The condensed X chromosome in the female).
2. Radioisotopes --- [^{3}H]-thymidine, [^{3}H]-uridine, [^{3}H]-adenosine, [^{14}C]-uridine, [^{111}In], [^{35}S], [^{51}Cr]-sodium chromate, [^{125}IudR] (Iododeoxiuridine), [^{3}H]-dextran sulphate, [^{32}P] (not used any more).

Radiolabels differentially label different classes of lymphocytes. They are detected either by organ counting (scintillation counting) or by autoradiography. (Ford, 1975; Ford, Smith, & Andrews, 1978; DeSousa,1981)

3. Isoantigens (Ly system) --- Among lymphocytes isoantigens are specific to T-cells (DeSousa, 1981).

4. Functional markers --- Functionally different classes of lymphocytes migrate differently. For example, when memory cells from a donor rat are passaged through an intermediate rat before being given to a receipient, lymphocytes from immunized donors which protect them from Listeria monocytogenes, say, do not migrate from blood to lymph in the intermediate (Ford, 1975).

2.2 THE EXPERIMENTAL RESULTS BEING MODELED.

As mentioned earlier, the distribution of labelled lymphocytes has been studied by many people. Some results were presented in figure 4 for rat TDL and fish (plaice) neural duct lymphocytes over time. At early instants of time (10 minutes and 30 minutes) a

considerable proportion of recovered trichloroacetic (TCA)-soluble radioactivity was present in the lungs and gills. By 24 hours, the majority of recovered radioactivity was present in the peripheral organs, i.e. spleen and lymph nodes in the rat and spleen and kidney lymphoid tissue in plaice (DeSousa,1981). Data are from Goldschneider & McGregor (1968) and Ellis & DeSousa (1974).

A more recent experiment along the same lines is that of Smith & Ford (1983). They try to correct some of the pitfalls of the previous studies and sample data more often. It is data from this experiment that will be modeled here.

2.2.1 THE EXPERIMENT OF SMITH & FORD (1983).

Smith & (late) Ford (1983) study the recirculating lymphocytes under conditions as close as possible to the physiological conditions. In the paper they report in effect three experiments: i) a survey of distribution of lymphocytes, ii) tempo of recirculation from blood to thoracic duct lymph, and iii) estimation of time taken for lymphocytes to cross HEV into LN. Only the one that is relevant to distribution will be

summarized here.

AO rats, adult male donors and adult female recipients were used. In vitro labeling of TDL was done with sodium-[^{51}Cr]-chromate, then passaged from blood to lymph in an intermediate rat to ensure using recirculating live lymphocytes. The results of the experiment are given in Table 1 and graphed in figure 5. Five rats were sacrificed at most of the 13 sample time points. (See section 4.1 for details). After removal of the 13 relevant organs (viz., blood, lungs, spleen, liver, right and left popliteal LN, coeliac LN, superficial cervical LN, deep cervical LN, mesenteric LN, peyer's patches, gut, and bone marrow) the results of scintillation counting were calculated as percentages of injected dose per organ and per gram of tissue. The results tabulated here are the means and standard deviations of activity per organ expressed as a

percentage of the injected dose.

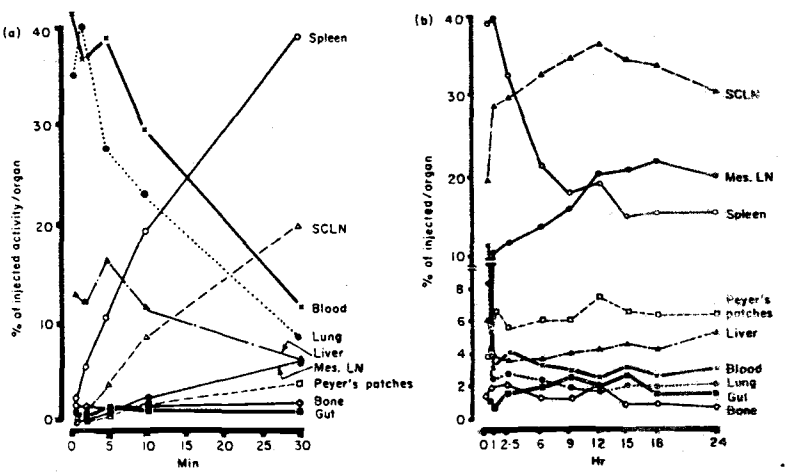


Figure 5. Means of the data from the experiment by Smith & Ford (1983). The values are expressed as % of injected dose per organ. (a) Covers 0-30 minutes and (b) 30 minutes to 24 hours. In (b) there are two scales on the ordinate.

Table 1

ORIGINAL DATA FROM SMITH & FORD (1983)
(mean + standard deviation)

% radioactive label (relative to injected dose) per organ

Time:	0	1	2	5	10	30	60	150	360	540	720	900	1080	1440
superficial cervical LN	0.00	0.18 +.06	0.24 +.11	1.02 +.68	2.20 +1.01	5.15 +1.18	7.46 +.76	7.71 +1.91	8.43 +1.72	8.97 +1.72	9.40 +1.13	8.88 +.43	8.75 +.99	7.83 +1.53
deep cervical LN	0.00	0.06 +.02	0.09 +.04	0.49 +.30	1.11 +.45	2.17 +.47	3.55 +.40	3.63 +.95	4.35 +.40	4.32 +.64	4.99 +.74	4.62 +.55	4.29 +.28	3.97 +.54
coeliac LN	0.00	ns	ns	0.09 +.07	0.15 +.06	0.41 +.15	0.60 +.13	0.60 +.13	0.84 +.17	0.71 +.18	0.94 +.16	0.84 +.14	0.68 +.17	0.81 +.13
mesenteric LN	0.00	0.14 +.05	0.17 +.07	0.86 +.65	1.66 +.65	4.91 +1.40	8.18 +1.43	9.32 +1.40	10.79 +1.32	12.65 +3.80	15.97 +3.25	16.34 +1.55	17.27 +2.48	15.62 +1.71
Peyer's patches	0.00		0.19	0.85 +.70	1.58 +.69	3.97 +1.07	6.68 +.96	5.73 +.50	6.16 +1.06	6.09 +.90	7.46 +1.53	6.68 +1.23	6.44 +.99	6.39 +1.27
small intestine	0.00	0.90 +.21	0.87 +.18	1.29 +.69	1.35 +.39	1.20 +.45	0.81 +.78	1.69 +.66	2.05 +.99	2.65 +1.35	1.99 +1.23	2.56 +1.47	1.54 +.51	1.72 +1.02
spleen	0.00	2.29 +1.79	5.31 +1.68	10.56 +2.64	19.51 +6.04	38.92 +5.45	39.41 +5.58	32.31 +3.42	21.32 +2.97	17.90 +3.72	19.14 +3.33	14.86 +3.22	15.48 +1.92	15.48 +.78
lung	0.00	35.50 +6.14	40.08 +6.13	27.63 +6.73	22.97 +4.87	8.46 +1.25	2.50 +.60	2.73 +.51	2.33 +.20	1.85 +.26	1.66 +.33	2.02 +.36	1.86 +.41	2.03 +.38
liver	0.00	12.84 +2.76	12.13 +2.82	16.24 +6.16	11.24 +2.70	6.22 +.83	3.92 +.83	3.66 +.39	3.66 +.58	4.04 +.39	4.11 +.45	4.56 +1.09	4.24 +.32	5.26 +.51
bone/tibia	0.00	1.15 +.21	1.15 +.21	1.36 +.49	1.61 +.44	1.81 +.69	2.26 +.50	2.23 +1.29	1.33 +.38	1.24 +.55	2.17 +1.22	0.72 +.34	1.01 +.50	0.73 +.15
blood/10ml	100.	41.47 +4.94	36.59 +1.80	38.91 +4.80	29.58 +5.34	11.64 +2.93	3.34 +1.15	4.06 +.88	3.25 +.34	3.06 +.52	2.41 +.56	3.13 +.92	2.46 +.58	3.04 +.65
R. popliteal LN	0.00				0.24 +.07	0.63 +.16	0.73 +.09	0.72 +.13	0.77 +.10	0.78 +.20	0.78 +.06	0.84 +.17	0.73 +.04	0.75 +.14
L. popliteal LN	0.00				0.06 +.02	0.16 +.06	0.21 +.04	0.23 +.06	0.22 +.06	0.28 +.08	0.33 +.06	0.28 +.05	0.25 +.02	0.23 +.03

Soon after injection (1 minute) 40% of the injected dose is in the lung, 40% in the blood, and 13% in the liver and very little elsewhere. After 2 minutes the radioactivity in the lungs follows that in blood. Till about 1/2 hour it falls sharply in both and substantially in the liver. In the spleen, and all the LN and peyer's patches the label steadily increases. Cell localization in gut and bone marrow seems flat from 1/2 hour on. At least in the spleen , LN, and peyer's patches the rate of cell entry is apparently directly proportional to their concentration in the blood as suggested by experiments on isolated organs (Smith & Ford, 1983). The network of interconnections for the system is given in figure 6. The superficial and deep cervical LN and the left and right popliteal LN were lumped together as subcutaneous LN (SCLN, total of about 550mg) a portion of which (about 350mg) are associated with efferent lymphatics and the rest (about 200mg)

2.3 MODELS OF RECIRCULATING LYMPHOCYTE DISTRIBUTION.

Data from the above Smith & Ford (1983) experiment has been used to model distribution behavior of lymphocytes. The deterministic models that have been developed are given below.

2.3.1 LINEAR TIME-INVARIANT MODEL (Mohler, Farooqi, & Heilig, 1984)

This model is based on the connectivity diagram in figure 6 above, each compartment being treated as obeying Fick's law. The output rate from each is assumed proportional to the amount of lymphocytes in that compartment. The model is:

$$\dot{x}_i(t) = \alpha_i x_{12}(t) - \beta_i g_i(t) x_i(t) \qquad i = 1,\ldots,5,7,9,10$$

$$\dot{x}_6(t) = \alpha_6 x_{12}(t) - \beta_6 g_6(t) x_6(t) + \beta_7 g_7(t) x_7(t)$$

$$\dot{x}_8(t) = \alpha_8 x_{12}(t) - \beta_8 g_8(t) x_8(t) + \beta_9 g_9(t) x_9(t) + \beta_{10} g_{10}(t) x_{10}(t)$$

$$\dot{x}_{11}(t) = \beta_4 g_4(t) x_4(t) - \beta_{11} g_{11}(t) x_{11}(t)$$

$$\begin{aligned}\dot{x}_{12}(t) = -[&\dot{x}_1(t) + \dot{x}_2(t) + \dot{x}_3(t) + \dot{x}_5(t) \\ &+ (\alpha_4 + \alpha_6 + \alpha_7 + \alpha_8 + \alpha_9 + \alpha_{10}) x_{12}(t) \\ &- \beta_6 g_6(t) x_6(t) - \beta_8 g_8(t) x_8(t) \\ &- \beta_{11} g_{11}(t) x_{11}(t)]\end{aligned} \qquad \ldots(2.1)$$

$$g_i(t) = 1.0 \qquad i = 1,\ldots,11 \qquad \ldots(2.2)$$

The subscripts are as follows:

1 = lungs
2 = bone marrow
3 = spleen
4 = liver
5 = subcutaneous LN (SCLN) with efferent lymphatics
6 = SCLN with other tissues
7 = miscellaneous tissues
8 = mesenteric LN
9 = gut
10 = peyer's patches
11 = coeliac LN
12 = blood

Written in vector form (2.1) has the same structure as (1.5) with B=0. The system is strongly connected. The system matrix A is a closed compartmental matrix and is irreducible (by Proposition A2.4). It is also essentially nonnegative. By propositions A2.1 and A2.2

the solution x(t) is nonnegative for $t \geqslant 0$ and by proposition A2.3 it is bounded. By proposition A2.5 the eigenvalues of A are nonpositive and by propositions A2.6 and A2.7 the spectral radius is zero. This means that we have a marginally stable system. It is an identifiable system (proposition A2.8) and the parameters are estimated using physiologically reasonable guesses to start with and varying these in a small neighboring interval by trial and error and trying to obtain as small a difference as possible between the estimates and the experimental data. The criterion used being the sum of the absolute values of the differences. The parameter values obtained are:

i	1	2	3	4	5	6	7	8	9	10	11
α_i	1.8	0.02	0.055	0.25	0.0045	0.002	0.15	0.003	0.015	0.004	
β_i	2.0	0.016	0.03	0.07	0.0002	0.03	0.004	0.001	0.005	0.0006	1.00

The model is sensitive to minor changes in parameters β_4, β_9, and β_{10}.

2.3.2 LINEAR TIME-VARIANT MODEL (Mohler, Farooqi, & Heilig, 1984)

The linear time-invariant model does not take into account time delays in the compartments. One way to approximate them is to use appropriate time-varying coefficients. For this case equation (2.1) is taken as before, and in (2.2) $g_i(t)$ are approximated by:

$$g_i(t) = \begin{cases} 1.0 & i = 1 \\ 1.0 - \exp(-t/\ ), & i = 2,4,\ldots,11 \\ \beta_3[1 + \frac{\gamma_3}{\beta_3}(1-\exp(-t/\tau_3))], & i = 3 \end{cases} \quad (2.3)$$

In vector form the system of equations become (1.6) with $\underline{u}=\underline{0}$. The system is strongly connected, closed, compartmental, and nonperiodic. By proposition A2.4, A(t) is irreducible; by proposition A3.2 $\underline{x}(t)$ is nonnegative for $t \geqslant 0$, and by proposition A3.1 the system is stable. It is an identifiable system (proposition A2.8), and the parameters are estimated as for the time-invariant case. The parameter values obtained are:

i	1	2	3	4	5	6	7	8	9	10	11
α_i	1.8	0.02	0.08	0.1	0.004	0.002	0.09	0.005	0.015	0.003	
β_i	1.7	1.00	0.0015	2.02	0.0005	0.05	0.01	0.005	0.05	0.003	1.00
γ_i			0.008								
$\tau_{i,min}$		540	60	400	500	200	200				20

2.3.3 LINEAR TIME-INVARIANT, TIME-DELAY MODEL (Mohler, Farooqi, & Heilig, 1986a; 1986b)

Instead of assuming capacitive delays as in the linear time-variant model above, discrete time delays are used in this case. Such delays may be interpreted as transit times through compartments. The model is a set of difference-differential equations:

$$\dot{x}_1(t) = \alpha_1 x_{12}(t) - \beta_1 x_1(t)$$

$$\dot{x}_i(t) = \alpha_i x_{12}(t) - \beta_i x_i(t) - \gamma_i x_i(t-\tau_i) \qquad i = 2,\ldots,5,7,9,10$$

$$\dot{x}_6(t) = \alpha_6 x_{12}(t) + \beta_7 x_7(t) + \gamma_7 x_7(t-\tau_7) - \beta_6 x_6(t) - \gamma_6 x_6(t-\tau_6)$$

$$\dot{x}_8(t) = \alpha_8 x_{12}(t) + \beta_9 x_9(t) + \gamma_9 x_9(t-\tau_9) + \beta_{10} x_{10}(t) + \gamma_{10} x_{10}(t-\tau_{10}) - \beta_8 x_8(t) - \gamma_8 x_8(t-\tau_8)$$

$$\dot{x}_{11}(t) = \beta_4 x_4(t) + \gamma_4 x_4(t-\tau_4) - \beta_{11} x_{11}(t) - \gamma_{11} x_{11}(t-\tau_{11})$$

$$\dot{x}_{12}(t) = -(\dot{x}_1(t) + \dot{x}_2(t) + \dot{x}_3(t) + \dot{x}_5(t)) - (\alpha_4+\alpha_6+\alpha_7+\alpha_8+\alpha_9+\alpha_{10}) x_{12}(t) + \beta_6 x_6(t) + \beta_8 x_8(t) + \beta_{11} x_{11}(t) + \gamma_6 x_6(t-\tau_6)$$

$$+ \gamma_8 x_8(t-\tau_8) + \gamma_{11} x_{11}(t-\tau_{11})$$

$$x_{13}(t) = x_5(t) + x_6(t)$$

where 13 = total SCLN,

τ_i = time-delay in compartment i

This system has properties similar to those above. The parameters are estimated using an algorithm in which integration is done numerically by Treanor's method and the optimization by Powell's technique (Powell,1964) with least squares criterion. It is a stiff system so that the use of ordinary fourth-order Runge-Kutta is not advisable. Treanor's method (Treanor, 1966) is used instead. The parameter values obtained are:

i	1	2	3	4	5	6	7	8	9	10	11
α_i	1.0	0.01	0.1	0.3	0.016	0.005	0.05	0.006	0.02	0.012	0.3
β_i	0.8	0.015	0.0007	0.3	0.0001	0.008	0.002	0.0006	0.03	0.001	0.7
γ_i		0.004	0.0075	0.0005	0.0027	0.0065	0.0004	0.0035	0.0005	0.0023	0.001
τ_i		250	60	600	180	150	300	150	150	180	180

In this case, the model is very sensitive to changes in the values of τ_4, τ_9, and τ_{10}, in particular the last two parameters.

2.3.4 NONLINEAR MODEL (Mohler, Farooqi, & Heilig, 1984)

The nonlinear model is based on a slightly different network than given in figure 6. All the lymph nodes (SCLN, mesenteric and coeliac LN) are lumped together to form a single compartment (# 5 in this case). Homogeneity of structure and function of LN is assumed. This simplies the migration network,the new version of which is given in figure 7. It is almost a mammillary system that is strongly connected and closed. The model is:

$$\dot{x}_1(t) = \alpha_1 x_{12}(t) - \beta_1 x_1(t)$$

$$\dot{x}_2(t) = \alpha_2(1-\gamma_2 x_2(t))x_{12}(t) - \beta_2(1-\exp(-t/\tau_2))x_2(t)$$

$$\dot{x}_3(t) = \alpha_3 x_{12}(t) - \beta_3[1+\frac{\gamma_3}{\beta_3}(1-\exp(-t/\tau_3))]x_3(t)$$

$$\dot{x}_4(t) = \alpha_4(1-\delta_4 x_4(t))x_{12}(t) - \beta_4 x_4(t) + \beta_3[1+\frac{\gamma_3}{\beta_3}(1-\exp(-t/\tau_3))]x_3(t)$$

$$\dot{x}_5(t) = \alpha_5 x_{12}(t) + \gamma_5(1-\delta_5 x_5(t))x_9(t) - \beta_5(1-\exp(-t/\tau_5))x_5(t)$$

$$\dot{x}_7(t) = \alpha_7 x_{12}(t) - \beta_7 x_7(t)$$

$$\dot{x}_9(t) = \alpha_9 x_{12}(t) + \gamma_9(1-\delta_9 x_9(t))x_7(t)x_9(t) - \beta_{10}(1-\exp(-t/\tau_{10}))x_{10}(t)$$

$$\dot{x}_{10}(t) = \alpha_{10} x_{12}(t) + \gamma_{10}(1-\delta_{10} x_{10}(t))x_{12}(t)x_{10}(t) - \beta_{10}(1-\exp(-t/\tau_{10}))x_{10}(t)$$

$$\dot{x}_{12}(t) = -(\dot{x}_1(t) + \dot{x}_2(t) + \dot{x}_3(t) + \dot{x}_4(t) + \dot{x}_5(t) + \dot{x}_7(t) + \dot{x}_9(t) + \dot{x}_{10}(t))$$

The parameters are estimated by trial and error as for the linear time-invariant case. The parameter values obtained are:

i	1	2	3	4	5	7	9	9	10
α_i	1.5	0.004	0.055	0.5	0.009	0.025	0.0015	0.0015	0.0011
β_i	2.0	0.016	0.0015	0.42	0.001	0.002	0.003	0.003	0.0006
γ_i		0.55	0.0075		0.0035		0.01	0.01	0.008
δ_i				0.055	0.047		1.0	1.0	0.16
$\tau_{i,min}$		360	150		720		360	360	540

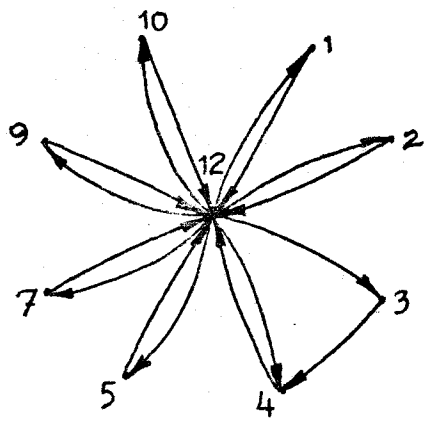
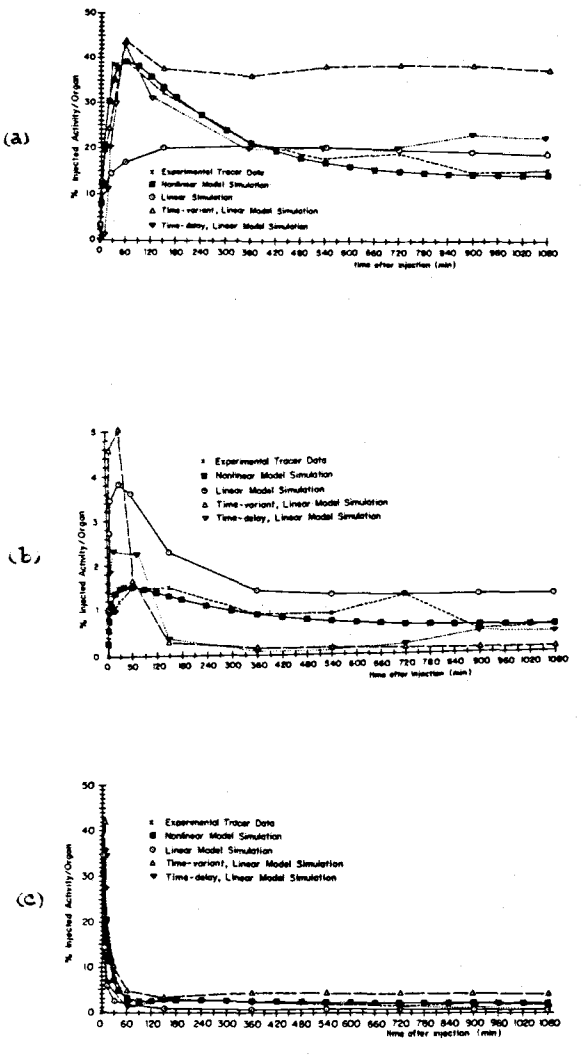


Figure 7. Connectivity diagram for the nonlinear model.

In the nonlinear model τ_2 and τ_{10} may be too large, and δ_5, δ_9, and δ_{10} for LN, gut, and PP may be too constraining. The states of the four models are compared in figures 8a to 8c (for spleen, bone marrow, and lungs) with each other and with the experimental data.



(a) Rat Spleen, Lymphocyte Response
(b) Rat Bone-Marrow, Lymphocyte Response
(c) Rat Lungs, Lymphocyte Response

Figure 8. States of the four models compared with the experimental data

CHAPTER 3

THE DISCRETE-TIME AND CONTINUOUS-TIME STOCHASTIC MODELS: THEIR DEVELOPMENT AND THEORETICAL ANALYSIS

3.0 INTRODUCTORY REMARKS.

Deterministic models were presented in the last chapter. In this chapter stochastic models will be developed for the distribution of recirculating lymphocytes in the body, parameters in the model explained, and the theoretical structure of the models analyzed.

In nature, generally processes occur as a combination of discrete and continuous events. All other things remaining constant the process of taking measurements or making observations is usually discrete. With such a process a data time series is generated,

inspite of the fact that the process being observed may be inherently continuous. That is, during observation, a continuous trajectory is sampled at discrete time points. In view of this, a discrete-time model and a continuous-time model, will be developed in that order. Before developing the models, some ideas which are common to both will be discussed, for example, the need for having stochastic models, how to represent randomness, and the description of the parameters in both models.

3.1 NEED FOR STOCHASTICITY.

Some researchers (Matis & Wehrly, 1979; Tiwari, 1979) have argued in favor of stochastic models because, according to their arguments, they are more general and realistic where deterministic models may be misleading. In the present case stochasticity is justified for several reasons. Some of them are uncertainties in the cellular microenvironment, uncertainty in the proportion of T- and B- cells and their properties, uncertainties in flow rates and physical properties of the vessels, and recognition of the fact that there is lack of information on the sum total of influences (which makes

it more realistic). There will be more justification for stochasticity when the parameters are described.

3.2 REPRESENTATION OF STOCHASTICITY.

Stochastic equations have been used in many areas of life sciences; e.g. population dynamics (Turelli, 1977; Weidlich & Haag, 1983), epidemics (Ludwig, 1974), genetics (Maruyama, 1983), and to describe neuronal behavior (Holden, 1983).

Stochasticity can be represented either in the form of stochastic difference equations or that of stochastic differential equations. Here stochasticity will be thought of as being generated by the coefficients. In stochastic difference equations, sequences of random variables are modeled. Such equations arise if i) a continuous-time model is approximated for computation on a computer, ii) some of the parameters in a deterministic model are treated as random processes, and iii) the system is perceived as inherently stochastic.

In the continuous case, stochastic differential equations may arise naturally. Two convenient

representations include Ito and Stratonovich (see Appendix 4) type of equations where the solution is a diffusion process. In the literature such models have been justified if:

i) the noise is wide-band;

ii) the time scales for the noise and the noise-free system dynamics are different, such that the noise is much faster; and

iii) a continuous Markov process is to be modeled.

Many examples involving stochastic differential equations are given in Arnold & Lefever (1981) and the special issue of Bulletin of Mathematical Biology (vol.45(4), 1983).

3.3 DESCRIPTION OF THE PARAMETERS.

Previously (Mohler, Farooqi, & Heilig, 1984; 1986a; 1986b) the parameters in the models of recirculating lymphocytes were simply referred to as directional permeabilities without explaining in detail what that meant. Such intuitive appeal to a phrase may be misleading. Here the factors influencing the parameters will be discussed in some detail.

Consider flow of lymphocytes in a single

compartment as in figure 9.

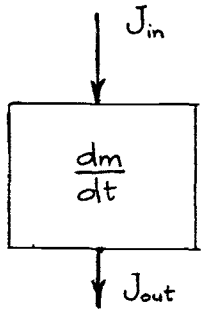


Figure 9. Fick's principle

Such a flow should satisfy Fick's principle, rheological properties of blood and those of lymphocytes (See section 2.1, esp. 2.1.1, 2.1.3, and 2.1.5). By Fick's principle (Rubinow, 1975)

$$\frac{dm}{dt} = J_{in} - J_{out} , \qquad \ldots(3.1)$$

where

m = mass, t = time,

J_{in} = mass influx rate of matter,

J_{out} = mass efflux rate of matter.

Since blood flow and WBC-vessel wall interaction are the most important factors in lymphocyte migration, there will be a digression through fluid dynamics of blood, which will be discussed next, en route to an

explanation of parameters in the model equations. The next four subsections are intended to provide a physical interpretation of the parameters.

3.3.1 HEMODYNAMICS.

Human blood constitutes a cell suspension in plasma, the latter being a solution which is 90% water by weight, 7% plasma protein, 1% inorganic molecules, and 1% other organic molecules (Guyton, 1976). Essentially all the cells are red blood cells (RBC or erythrocytes), with all white blood cells (WBC or leukocytes) making up < (1/600)-th of the total cellular volume, and platelets < (1/800)-th of the cellular volume. Under ordinary conditions RBC occupy about 50% of blood volume. They are small and number about $5\times10^{6}/mm^{3}$. Normally WBC are about 5000-8000/mm^{3}, and platelets 250,000-300,000/mm^{3}. Plasma behaves like a Newtonian viscous fluid with coefficient of viscosity = 1.2 cP (centi-Poise), but the whole blood is non-Newtonian because the blood viscosity varies with the hematocrit (% of total volume of blood occupied by cells). (Fung, 1981)

If the width of the channel in which blood flows

is much greater than RBC diameter, blood can be considered a homogeneous fluid. In the human body there are about 10^{10} blood vessels with diameter approximately equal to RBC diameter, ranging between 4-10μm. These are the capillaries, in which the RBC are squeezed and deformed, and move in a single file. In this case it is more useful to consider blood as a nonhomogeneous fluid, with two phases, one phase being the RBC, the other being the plasma.

As just noted the non-Newtonian characteristics of human blood are due to blood cells. How these cells move, especially RBC, is an important issue. It has been observed that human RBC tend to form aggregates, called rouleaux, because of the presence of fibrinogen and globulins in the plasma. The smaller the blood flow rate the more prevalent the rouleaux. With increase in shear rate, rouleaux tend to break up, and the blood viscosity reduces. Tumbling of the rouleaux while flowing adds to disturbance in flow and complicates the cell motion. Rouleaux do not form in all animals. RBC are deformable and when single they tend to be aligned with the streamlines.

The hematocrit (% cell vol./blood vol.) in normal blood is quite high --- about 45 in large vessels, and

25 in small ones. At such concentrations, no one cell can act alone. Goldsmith (1972) as quoted in Fung (1981) has observed that due to this crowding:

i) The velocity profile in a channel is no longer parabolic as in Poiseuille flow (See section 3.3.2),

ii) RBC deformation is more than can be attributed to shear alone, and

iii) Cell paths exhibit erratic displacements in a direction normal to the flow. This is because of frequent encounters of a cell with neighboring cells. The cell path therefore, shows features of a random walk.

It has been pointed out previously that RBC tend to move toward the tube axis during flow. This leaves a marginal layer of plasma, whose width increases with increase in the shear rate. This layer is relatively free of RBC. Unlike RBC, the WBC tend to move toward the walls (See section 2.1.5).

In the next two sections homogeneous flow in big vessels and nonhomogeneous flow in small ones will be discussed. After that there will be a brief discussion of factors involved in WBC interaction with vessel walls.

3.3.2 FLOW IN BIG VESSELS.

If blood flow is assumed to be a steady, laminar flow in a long, rigid, circular, cylinderical tube, then such a flow is governed by Hagen - Poiseuille Law (Fung, 1984).

$$\dot{Q} = \frac{\pi d^4}{128\mu} \cdot \frac{\Delta p}{L}, \qquad \ldots(3.2)$$

where

$\dot{Q}$ = flow rate or volume flow rate,

d = vessel diameter,

μ = fluid (blood) viscosity,

L = length of vessel segment over which pressure drop is Δp,

Δp = pressure drop along L.

3.3.3 MICROCIRCULATION (Fung, 1981; Fung, 1984)

Reynold's number (the ratio of inertial to viscosity forces) tends to 1.0 at the level of terminal arteries. Further downstream, in the arterioles, capillaries, and venules it tends to become less than 1.0. Thus inertial forces become less important and

flow is determined by a balance of viscosity forces and pressure gradient at this level. Features unique to microcirculation are:

i) Small Reynold's number (<<1.0),

ii) Blood cells behave individually, not as a group,

iii) Exchange of fluid and other matter occurs between blood and tissue surrounding the blood vessels, and

iv) Flow is regulated locally by the smooth muscle of the microvasculature

A striking feature of capillary circulation is the continuous variation of blood flow. Changes occur in velocity, direction of movement, and number of capillaries with active circulation. Capillary blood flow is nonhomogeneous and unsteady, a major part being due to heartbeat. Sometimes RBC rush by, and at other times there are no RBC at all. Thus the velocity is unsteady and so is the hematocrit. Interaction between cells and tube walls becomes significant. Flexibility of RBC and active migration of WBC play a role.

Apparent coefficient of viscosity of blood in microvessels can be greatly increased under the following conditions:

i) Existence of large WBC or exceptionally large

RBC (with diameters greater than that of the capillary blood vessel). The vessel may become occluded by these cells.

ii) The smooth muscle in the arterioles or in the sphinctors of the capillaries may contract so that the diameters of these vessels are greatly reduced, causing the effect of i) to take place. This contraction may be initiated by nerves, metabolites, or by mechanical stimulation.

iii) The WBC have a tendency to adhere to the blood vessel wall. If they do, they increase resistance to blood flow. Thrombocytes may be activated, causing clotting and increasing resistance further.

iv) Cell flexibility may be changed. Hardening of RBC, as in sickle cell anemia, increases the coefficient of viscosity of the blood.

The effects ii) and iii), besides controlling apparent viscosity, effectively control the vessel diameter. Since diameter appears in fourth power in equation (3.2), its importance is obvious.

3.3.4 FORCE OF INTERACTION OF LEUKOCYTES AND VASCULAR ENDOTHELIUM (Fung, 1984).

The flow behavior of RBC and WBC is different. RBC do not stick to the endothelium unless they are damaged. WBC, however, often stick to the endothelial wall or roll slowly on it, while plasma and RBC rush by them. The resultant shear force S (dynes) imparted to WBC by blood flow can be expressed as a dimensionless ratio $S/(V_m \mu d_c)$ which is a function of other dimensionless constants:

$$\frac{S}{V_m \mu d_c} = f\left(\frac{d_c}{d_t}, \frac{V_c}{V_m}, \frac{V_m d_c \rho}{\mu}, H\right) \qquad \ldots(3.3)$$

where

d_c = diameter of WBC considered as a sphere,

d_t = diameter of blood vessel,

V_m = max. velocity of undisturbed flow in the blood vessel,

V_c = linear velocity of centroid of the WBC,

μ = plasma coefficient of viscosity,

ρ = plasma density,

H = hematocrit.

Noting that Reynold's number $N_R = \rho V_m d_c / \mu$ and refering

to the ratio $S/V_m \mu d_c$ as shear coefficient C_s, we have

$$C_s = f\left(\frac{d_c}{d_t}, \frac{V_c}{V_m}, N_R, H\right) \qquad \ldots(3.4)$$

C_s is strongly dependent on H. Since H is stochastic in vivo (see section 3.3.3), the shear force fluctuates with time.

A WBC adhering to a vessel wall is subjected to shear stress on its surface by a number of factors: by the plasma and the RBC, by pressure variations in the flowing blood, by a shear stress at the interface of the WBC and the endothelium, by a variable normal stress on the interface, and by a body force due to acceleration and gravity. The mean rolling velocity of the WBC is only about 4% of the mean blood flow velocity in the venule. With such a small rolling velocity, the flow pattern around the rolling WBC should be almost the same as that around a stationary cell.

Using a geometrically and dynamically similar system it has been shown experimentally that for the shear force:

$$S \propto N_R, \; S \propto \frac{d_c}{d_t}, \; S \propto H, \; S \propto V_m \qquad \ldots(3.5)$$

Thus, the forces acting on WBC are shear and the

effective frictional drag. Velocity, being dependent on shear and drag, is a function of x, the distance along the vessel, as both the forces are. Thus acceleration is a function of x. Because of different accelerations at different points (x), the concentrations (C) are different at different x, hence there is a concentration gradient ($\partial C/\partial x$) and by Fick's first law of diffusion a current ($J = -DA(\partial C/\partial x)$ or)

$$J = \alpha \cdot \Delta C$$

exists, where the coefficient

$$\alpha = f(D, A, S, F_{drag}, x), \qquad \ldots(3.6)$$

D = diffusion constant,

A = cross-sectional area of the vessel.

Thus if the compartment in Fig 9 is thought of as a blood vessel, J_{in} and J_{out} in (3.1) may be written in terms of cell concentrations, the coefficients being interpreted as functions of various factors mentioned in (3.6). The shear S in (3.6) is in turn a function of the influences pointed out in (3.4).

3.4 DEVELOPMENT OF THE DISCRETE-TIME MODEL.

In this section a discrete-time model will be presented for recirculating lymphocytes. Data from the Smith and Ford (1983) experiment discussed in section 2.2 will be used as was done for the deterministic models in section 2.3. A mathematical analysis of systems which have a structure similar to the model developed here is given in Appendix 5.

For our purposes a simplified system with 7 compartments will be used, instead of the original 13 compartments. Recognizing the importance of blood, lungs, spleen and bone marow they have been kept as separate compartments. All the others have been lumped into three compartments, assuming homogeneity of structure and function within a compartment: i) lymphoid tissue like liver, gut, peyer's patches and nonlymphoid miscellaneous tissues as "other tissues", ii) the LN (SCLN, MLN, CLN, etc) draining the other tissues compartment as "LN-a", and iii) all the other LN as

"LN-b". The new network is shown in figure 10.

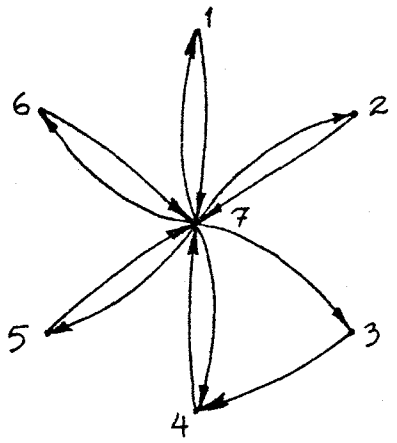


Figure 10. Connectivity diagram for the 7 compartment model. (Key: 1=Bone marrow, 2=Spleen, 3="Other tissue", 4=LN-a, 5=LN-b, 6=Lungs, 7=Blood.)

Discussion of blood flow in the previous section was concerning blood vessels, both large and small. Organs or the compartments just mentioned may be thought of as vessels which sometimes behave as large and sometimes as microvessels (as in trapping). Thus they each possess an effective diameter and an effective viscosity which are functions of its vasculature and the physiological microenvironment prevailing at that particular time. For any compartment the label present

at any instant depends on the total amount that enters and the total amount that leaves that compartment. The amount that leaves is proportional to the concentration in that compartment. The amount entering is the sum of the amounts coming from various compartments, each being proportional to label concentrations in the source compartment. The "constants" of proportionality are taken as random variables which have a deterministic component and a stochastic component. In this way equations similar to (3.1) hold for each compartment and the parameters can be given similar interpretation as before but now the parameters refer to compartments rather than to blood vessels. Flow rates in different organs in rat are not available, for the most part because it has been difficult to measure them up to now in an animal of that size. Because of this presence of inherent stochasticity and the lack of precise knowledge of various variables the model equations in

discrete-time are:

$$x_i(n+1) = (1-\alpha_i)x_i(n) - \beta_i(n)x_i(n) + a_{i7}x_7(n) + b_{i7}(n)x_7(n) + \varepsilon_i(n+1), \qquad i = 1,2,3,5,6$$

$$x_4(n+1) = \alpha_3 x_3(n) + \beta_3(n)x_3(n) + (1-\alpha_4)x_4(n) - \beta_4(n)x_4(n) + a_{47}x_7(n) + b_{47}(n)x_7(n) + \varepsilon_4(n+1)$$

$$\begin{aligned} x_7(n+1) = {} & \alpha_1 x_1(n) + \beta_1(n)x_1(n) + \alpha_2 x_2(n) \\ & + \beta_2(n)x_2(n) + \alpha_4 x_4(n) + \beta_4(n)x_4(n) \\ & + \alpha_5 x_5(n) + \beta_5(n)x_5(n) + \alpha_6 x_6(n) \\ & + \beta_6(n)x_6(n) \\ & + [1 - (a_{17}+a_{27}+a_{37}+a_{47}+a_{57}+a_{67})]x_7(n) \\ & - (b_{17}(n)+b_{27}(n)+b_{37}(n)+b_{47}(n)+b_{57}(n) \\ & + b_{67}(n))x_7(n) - \varepsilon_7(n+1), \end{aligned} \qquad \ldots(3.7)$$

where

$$\varepsilon_7(n+1) = \sum_{i=1}^{6} \varepsilon_i(n+1) \qquad \ldots(3.8)$$

$x_i(n)$ = state of compartment i at time instant n, being the number of lymphocytes in compartment i expressed as a % of the total number in the system as measured

by the presence of radioactive label.

α_i = deterministic part of transfer parameters (leaving i), a_{i7} is portion of α_7 going from 7 to i. α_i (and a_{i7}) scales the % activity in compartment i (or 7) at instant n (dimensionless).

$\beta_i(n)$ = stochastic part of transfer parameters (leaving i), $b_{i7}(n)$ is the portion of $\beta_7(n)$ going from 7 to i. $\{\beta_i(n)\}$ are i.i.d. and constitute multiplicative noise (dimensionless).

$\varepsilon_i(n)$ = additive noise, added at the input and/or output of the compartment, $\{\varepsilon_i(n)\}$ are i.i.d. (dimensions of % activity).

$\{\beta_i(n)\}$ and $\{\varepsilon_i(n)\}$ are independent.

The subscripts are as follows:

1 = bone marrow, 2 = spleen,
3 = other tissue, 4 = LN-a,
5 = LN-b, 6 = Lungs,
7 = blood.

In vector form

$$\underline{x}(n+1) = A\,\underline{x}(n) + \beta(n)\,\underline{x}(n) + \underline{\varepsilon}(n+1) , \qquad \ldots(3.9)$$

where

$$\underline{x}(n) = [x_1(n) \ \ldots \ x_7(n)]'$$

$$\underline{\varepsilon}(n) = [\varepsilon_1(n) \ \ldots \ \varepsilon_7(n)]'$$

' stands for transposition.

$$A = \begin{bmatrix} 1-\alpha_1 & & & & & & a_{17} \\ & 1-\alpha_2 & & & \phi & & a_{27} \\ & & 1-\alpha_3 & & & & a_{37} \\ & \phi & \alpha_3 & 1-\alpha_4 & & & a_{47} \\ & & & & 1-\alpha_5 & & a_{57} \\ & & & & & 1-\alpha_6 & a_{67} \\ \alpha_1 & \alpha_2 & 0 & \alpha_4 & \alpha_5 & \alpha_6 & 1-\alpha_7 \end{bmatrix} \quad \ldots(3.10)$$

$$\alpha_7 = \sum_{i=1}^{6} a_{i7} \quad \ldots(3.11)$$

$$\beta(n) = \begin{bmatrix} -\beta_1(n) & & & & & & b_{17}(n) \\ & -\beta_2(n) & & & \phi & & b_{27}(n) \\ & & -\beta_3(n) & & & & b_{37}(n) \\ & \phi & \beta_3(n) & -\beta_4(n) & & & b_{47}(n) \\ & & & & -\beta_5(n) & & b_{57}(n) \\ & & & & & -\beta_6(n) & b_{67}(n) \\ \beta_1(n) & \beta_2(n) & 0 & \beta_4(n) & \beta_5(n) & \beta_6(n) & -\beta_7(n) \end{bmatrix} \quad \ldots(3.12)$$

$$b_{i7}(n) = \frac{a_{i7}}{\alpha_7}\beta_7(n) \quad \text{such that} \quad \beta_7(n) = \sum_{i=1}^{6} b_{i7}(n). \quad \ldots(3.13)$$

In the above model the state of a compartment is the number of lymphocytes in it expressed as a percentage of the total number of lymphocytes in all the compartments. This total number is equivalent to the number of injected lymphocytes, assuming no births and deaths, and no loss of label. Experimentally the number of cells in any compartment is measured by the amount of

radiolabel activity. The 7×7 matrix A is constant and stochastic (in the sense of Appendix 1). The 7×7 matrix $\beta(n)$ is compartmental (in the sense of section 1.1.1.4) and is interpreted as the random component in (3.6). $\underline{\varepsilon}(n)$ is a random 7-vector. Presence of $\varepsilon_i(n)$ in (3.7) may be justified by changes in the same variables as for $\beta_i(n)$, but this time these are due to intercompartmental stochasticity rather than intracompartmental randomness (as for $\beta_i(n)$). Also $\varepsilon_i(n)$ may mimic the presence of different types of immunities (see sections 1.2 and 2.1) and other physiological factors, and the random variation in different organisms within the same species.

Equation (3.9) can be viewed as a system of difference equations with both slope and intercept random. The same system may be thought of as a Markov chain generated by two stochastic processes. In other words, it is a Markov chain in a random environment, but such processes will not be discussed here. The second term on the right-hand side of (3.9) causes the structure to be similar to that of bilinear time series.

A fairly detailed discussion of bilinear time series with one and two inputs is given in Appendix 5. A bilinear time series with two inputs has sometimes

been called random coefficient autoregression. The discussion in Appendix 5: includes assumptions made in analyzing the series, recursive expressions for the solution and the first two moments, stability, stationarity, existence and convergence of moments, and asymptotic properties of least squares and maximum likelihood estimators. Here, only the biological interpretation of the results in the appendix will be presented.

It is assumed that, in (3.9), A is a constant matrix, $\beta(n)$ is a matrix such that $E[\beta(n)] = 0$ and $E[\beta(n)\otimes\beta(n)] = C_\beta$, and $\underline{\varepsilon}(n)$ is such that $E[\underline{\varepsilon}(n)] = 0$ and $E[\underline{\varepsilon}(n)\underline{\varepsilon}'(n)] = G$. $\{\beta_i(n)\}$ and $\{\varepsilon_i(n)\}$ are independent. $\otimes$, the Kronecker product, is defined in section A5.1. Equation (3.9) may be used to find the solutions (A5.5) and (A5.11). Stability can be thought of as the ability to reach an equilibrium value independent of the initial condition. For a more precise statement of second-order stability see section A5.3. In the case of the system under discussion it depends on two second order terms: one based on the determininstic coefficients, $A\otimes A$ and the other based on the interaction term, the variation in A, C_β. The biological interpretation is given in the biological corollary 3.1 (corresponding to proposition A5.1).

BIOLOGICAL COROLLARY 3.1. The number of recirculating lymphocytes in any compartment reaches an equilibrium value if the variation in that number reaches an equilibrium and vice versa. This happens independently of the number of recirculating lymphocytes present initially. The variation in the number of lymphocytes depends on the deterministic parameters of the system and the variation in these parameters within the compartment.

For the system (3.9) constancy of mean and variance implies second-order stationarity (see corollary A5.1). Precise results are in section A5.4. Biological corollaries 3.2 and 3.3 correspond to propositions A5.2 and A5.3 respectively.

BIOLOGICAL COROLLARY 3.2 If all the disturbances are independent of each other, then the number of lymphocytes have a constant mean and variance if the variation in the number reaches an equilibrium and vice versa.

BIOLOGICAL COROLLARY 3.3. The mean number of lymphocytes and the variation around it described in Biological Corollary 3.2 have unique values.

The condition for second-order stability and

second-order stationarity is the same. They are related as seen in proposition A5.4. In fact they are two versions of the same concept; one (stability) refering to the ability to reach equilibrium independent of the initial condition, and the other (stationarity) emphasizing the values reached. The following reflects this relationship.

BIOLOGICAL COROLLARY 3.4. If the number of lymphocytes in any compartment in figure 10 reaches an equilibrium independent of the initial conditions then the distribution of lymphocytes in that compartment has a constant mean and variance. With the passage of time the equilibrium value of the number of cells and the constant mean number of cells coincide.

Strict stationarity reflects time-invariance of distributions (see section A5.6). The following corollary reflects this idea corresponding to proposition A5.5.

BIOLOGICAL COROLLARY 3.5. If the noises are independent and identically distributed amongst themselves and over time, then in any compartment the distribution of lymphocytes which has constant mean and variance as described above becomes asymptotically time-invariant.

3.5 SUMMARY OF BIOLOGICAL IMPLICATIONS.

A summary of the results presented as biological corollaries above is given here. If the variation in the number of recirculating lymphocytes in any compartment reaches an equilibrium which does not depend on the number of cells present initially in the compartment then the mean number of cells also reaches an equilibrium. This means that at equilibrium the distribution of these cells in that compartment has attained a unique constant mean and standard deviation. When the random perturbations in the RLP are independent and identically distributed, the necessary and sufficient condition for the lymphocyte distribution in any compartment to have a constant mean and standard deviation at equilibrium is that the variation in the number of lymphocytes there be stable. Under these conditions the distribution is also asymptotically time-invariant. This implies that the number of recirculating lymphocytes can be characterized in terms of their population distributions in different compartments.

3.6 DEVELOPMENT OF THE CONTINUOUS-TIME MODEL.

As mentioned before a time series is generated when a continuous process is sampled at discrete time points. Up to now a discrete-time model and its properties were presented. In the remaining portion of the chapter a corresponding continuous-time model and relevant theoretical analysis will be discussed.

Physiological reasoning is similar to that in section 3.3 and 3.4 and still 7 compartments will be modeled. The mathematical reasoning follows that of Zuev (see Marchuk et.al., 1985a; Marchuk et. al., 1986) very closely. The deterministic model would be a system of ordinary differential equations

$$\frac{dx(t)}{dt} = f(x(t), \theta), \quad t\ [0,\tau] , \qquad ...(3.14)$$

where $x(t) \in \mathbb{R}^n$ is a state vector,

$\theta \in \mathbb{R}^m$ is the vector of parameters,

$x(0)$ is the initial state.

The undisturbed state of the system corresponds to

$$x_t(\bar{\theta}) = x(t,\theta) , \qquad ...(3.15)$$

while the disturbed state is realized by using

$$\theta = \bar{\theta} + \delta\theta(t) \qquad \ldots(3.16)$$

in (3.14). Here $\delta\theta(t) = \delta\theta_t \in \mathbb{R}^m$ is a function of time. Consider the i-th real trajectory

$$x_t^i = x_t(\theta + \delta\theta_t^i)$$
$$t\in[0,\tau],\ x_t^i \in \mathbb{R}^n.$$

The set $X^i = \{x_t^i,\ t\in[0,\tau],\ i = 1,2,\ldots,l\}$ is the realization of a stochastic process. Thus the stochastic model for the description of the disturbed state of the system is

$$\frac{dx_t}{dt} = f(x_t, \bar{\theta}+\delta\theta_t), \quad t\in[0,\tau]\ . \qquad \ldots(3.17)$$

Assume the perturbations to be small and rewrite (3.16) using x_t^ε to denote the disturbed state.

$$\frac{dx_t^\varepsilon}{dt} = f(x_t^\varepsilon, \bar{\theta}+\delta\theta_t)\ , \qquad \ldots(3.18)$$

where x_t^ε is fixed, $\varepsilon > 0$ is small. The random disturbances in (3.18) will be considered small if with small $\varepsilon > 0$,

$$\lim_{\varepsilon\to 0} P\{\sup_{0\le t\le\tau} |x_t^\varepsilon - x_t(\bar{\theta})| > \delta\} = 0 \qquad \ldots(3.19)$$

for any $\delta > 0$. Equation (3.19) may be interpreted as the formal statement of the assumption that the observed trajectories have a common state $x_t(\bar{\theta})$, i.e. $x_t(\bar{\theta}) = Ex_t^\varepsilon$. Assume that the random deviations $(x_t^\varepsilon - x_t(\bar{\theta}))$ are short-lived, in other words they are caused by a fast random variable. Thus in (3.17) assume $\delta\theta_t = \xi_{t/\varepsilon}$ where ξ_t is a stochastic process with values in $\mathbb{R}^m$, $\varepsilon > 0$ and small. Therefore

$$\frac{dx_t^\varepsilon}{dt} = f(x_t^\varepsilon, \bar{\theta} + \xi_{t/\varepsilon}) \qquad t\in[0,\tau] \ . \qquad ...(3.20)$$

The parameter ε takes into accounts the division of variables into fast and slow components. The disturbances have been assumed to be random i.e. $E[\xi_t] = 0, \forall t$. Also, since they are fast, for arbitrary $\tau > 0$, $\delta > 0$ and $x_t^\varepsilon \in \mathbb{R}^n$

$$\lim_{\varepsilon \to 0} P\{\left| \int_t^{t+\tau} f(x_t^\varepsilon, \bar{\theta} + \xi_{t/\varepsilon})ds - \tau f(x,\bar{\theta})\right| > \delta\} = 0 \ . \qquad ...(3.21)$$

In such a case it can be shown that with

$$\sup_t E|f(x_t, \xi_t)|^2 < \infty \quad \text{for any } \tau>0,\ \delta>0 \ ,$$

equation (3.18) holds. The process ξ_t may be considered to fulfill the strong mixing condition. Taking this, together with certain other assumptions (see proposition A4.3), it can be shown that the normalized difference

$$\zeta_t^\varepsilon = (x_t^\varepsilon - x_t(\bar{\theta}))/\sqrt{\varepsilon}$$

converges weakly, as $\varepsilon \longrightarrow 0$, in $[0,\tau]$ to a Gauss-Markov process ζ_t° satisfying

$$d\zeta_t^\circ = A(x_t(\bar{\theta}),\bar{\theta})\zeta_t^\circ dt + dW_t^\circ, \quad \zeta_0^\circ = 0 , \quad \ldots(3.22)$$

For the structure of A and for other details see Appendix 4.

Writing the deviations as

$$\delta x_t = (x_t^\varepsilon - x_t(\bar{\theta})) = \sqrt{\varepsilon}\,\zeta_t^\varepsilon , \quad \ldots(3.23)$$

and using (3.23) in (3.22) yields,

$$d\delta x_t = A(x_t(\bar{\theta}),\bar{\theta})\delta x_t dt + \sqrt{\varepsilon}\, dW_t^\varepsilon . \quad \ldots(3.24)$$

Assuming $C = \mathrm{cov}(W_t^\circ, W_{t+s}^\circ) = [c_{ij}]$ and considering the right hand side to be linear in θ, (3.24) can be written as

$$d\delta x_t = A(x_t(\bar{\theta}),\bar{\theta})\delta x_t dt + G dW_t , \quad \ldots(3.25)$$

where $G = [\sqrt{\varepsilon}\, c_{ij}]$ and W_t is a Wiener process with covariance matrix as Idt, I being the identity matrix. The undisturbed compartmental model can be written from (3.14) as:

$$dx_t = A(x_t(\bar{\theta}),\bar{\theta})x_t dt \ . \qquad \ldots(3.26)$$

Equation (3.25) describes the deviation in (3.26). Adding (3.25) and (3.26)

$$d(x_t + \delta x_t) = A(x_t(\bar{\theta}),\bar{\theta}).(x_t + \delta x_t)dt + GdW_t \ . \qquad \ldots(3.27)$$

Using $y_t = x_t + \delta x_t$ in (3.27)

$$dy_t = A(x_t(\bar{\theta}),\bar{\theta}).y_t\, dt + GdW_t \ . \qquad \ldots(3.28)$$

Here matrix A(.,.) is a function of the undisturbed state, but in general it can be under the influence of random disturbances so that we may rewrite (3.28) as

$$dy_t = A(y_t(\theta),\theta).y_t\, dt + G(y_t(\theta),\theta)dW \ . \qquad \ldots(3.29)$$

3.7 THE SOLUTION AND ITS PROPERTIES.

After enlisting the relevant assumptions, the solution of (3.29) and its properties will be presented. There will be a brief mention of complexity of the solution. Properties related to long-term behavior, stability and stationarity, will be taken up just as for the discrete model. Relation to diffusions and their recurrence properties will also be mentioned.

Unlike the discrete-time model here results on the general structure will be discussed and the model equations treated as a special case of the general equation (3.30) so that the results hold for this also.

3.7.1 ASSUMPTIONS.

Rewrite (3.29) as

$$dx_t = f(t,x_t)\, x_t\, dt + G(t,x_t)\, dW_t \ . \qquad ...(3.30)$$

Following will be assumed as related to the continuous-time model which is a special case of (3.30).

$$x_{t_o} = c, \ t_o \leq t \leq \tau < \infty \ ,$$

where $x_t \in \mathbb{R}^n$, $t \in [t_o, \tau]$, W_t is an m-dimensional Wiener process, $f \in \mathbb{R}^n$, G ($n \times m$ matrix) $\in [t_o, \tau] \times \mathbb{R}^n$. x_t is nonanticipating for $t \in [t_o, \tau]$. c is a random variable independent of $W_t - W_{t_o}$.

3.7.2 THE SOLUTION AND ITS COMPLEXITY

For conditions of existence and uniqueness of the solution of a stochastic differential equation (SDE) see Arnold (1974) or any other standard text on stochastic differential equations. The general linear stochastic differential equation of interest here can be written as

$$dx_t = (A(t)x_t + a(t))dt + \sum_{i=1}^{m} (B_i(t)x_t + b_i(t))dW_t^i , \qquad ...(3.31)$$

where

$x \in \mathbb{R}^n$; $a(t), b(t) \in \mathbb{R}^n$; $A(t), B(t) \in \mathbb{R}^{n \times n}$;

$W_t = (W_t^1, \ldots, W_t^m)' \in \mathbb{R}^m$ is a Wiener process.

Even when $a(t) \equiv b(t) \equiv 0$, and $A(t) \equiv A$ and $B_i(t) \equiv B_i$

$$dx_t = Ax_t dt + \sum_{i=1}^{m} B_i x_t dW_t^i , \qquad ...(3.32)$$

the solution of (3.30), x_t, cannot in general be given explicitly in a simple form. Only when the matrices $A, B_1, \ldots, B_m$ commute

$$x_t = \exp((A - \sum_{i=1}^{m} B_i^2/2)(t-t_o) + \sum_{i=1}^{m} B_i(W_t^i - W_{t_o}^i)) \quad \ldots(3.33)$$

When they do not commute the solution is more complicated. It is well known that the complexity of the solution of (3.30) depends on the vector fields generated by $g^1(t,x), \ldots, g^m(t,x)$ comprising the columns of $G(t,x)$. Under certain conditions the solution of (3.31) can be written explicitly, but the expression is quite complicated. For details see Kunita (1980, 1981).

Although in some cases the solution of (3.30) may be available, generally it is not in a convenient form and would be very cumbersome if used in a simulation or in estimation. Fortunately other ways are available to study certain statistics of the nonlinear solution.

3.7.3 FIRST AND SECOND MOMENTS OF THE SOLUTION.

The relations that the first two moments of the

solution of (3.31) must satisfy are given in Appendix 4 (see proposition A4.2). These relations ((A4.4) for the first moment and (A4.5) for the second moment) are ordinary differential equations and can be handled in the usual way.

3.7.4 LONG-TERM BEHAVIOR OF THE SOLUTION.

3.7.4.1 STABILITY OF THE SOLUTION.

Consider (3.31) with continuous coefficients with respect to t. Assume that the existence and uniqueness conditions are satisfied and that with probability one, c is a constant. Stability of the first and second moments reduces to the stability of equations (A4.4) and (A4.5).

3.7.4.2 SDE's AS DIFFUSIONS.

Under proper assumptions (Thm 9.3.1 of Arnold, 1974) SDE's can be thought of as diffusions. Autonomous SDE's can always be taken as homogeneous diffusions.

For more details refer to Appendix 4, section A4.4.

3.8 THE CONTINUOUS-TIME MODEL EQUATIONS AND THOSE USED IN ESTIMATION.

For the lymphocyte distribution model, equation (3.31) is assumed with $a(t) \equiv b(t) \equiv 0$ and $m = 7$, the number of compartments. Further it is assumed that $A(t) \equiv A$, $B_i(t) \equiv B_i$, i.e. they are constant. In other words a homogeneous, autonomous system analogous to the discrete model is obtained as

$$dx_t = A\, x_t\, dt + \sum_{i=1}^{7} B_i\, x_t\, dW_t^i \qquad \ldots(3.34)$$

Additive noise is assumed zero for the time being. As the analytical solution of this is rather unwieldy when the Lie algebra generated by $A, B_1, \ldots, B_7$ is not Abelian, (A4.4) is used to estimate A, and (A4.5) to estimate B_i, $i = 1,\ldots,7$. In the present case (A4.4) and (A4.5) reduce respectively to

$$\dot{m}_t = A\, m_t \qquad \ldots(3.35)$$

and

$$\dot{P}(t) = AP(t) + P(t)A' + \sum_{i=1}^{7} B_i P(t) B_i' \qquad \ldots(3.36)$$

The matrices A and B_i, i = 1,...,7 are as follows:

$$A = \begin{bmatrix} -\alpha_1 & & & & & & a_{17} \\ & -\alpha_2 & & & \phi & & a_{27} \\ & & -\alpha_3 & & & & a_{37} \\ & \phi & \alpha_3 & -\alpha_4 & & & a_{47} \\ & & & & -\alpha_5 & & a_{57} \\ & & & & & -\alpha_6 & a_{67} \\ \alpha_1 & \alpha_2 & 0 & \alpha_4 & \alpha_5 & \alpha_6 & -\alpha_7 \end{bmatrix} \qquad \ldots(3.37)$$

$$\alpha_7 = \sum_{i=1}^{6} a_{i7}$$

A is thus irreducible and compartmental (essentially nonnegative). B_i, i = 1,...,6 are 7×7 with only 2 non-zero elements in column i; all the other elements are zero:

$$B_1 = \begin{bmatrix} -\beta_1 & \\ 0 & \\ 0 & \\ 0 & \phi \\ 0 & \\ 0 & \\ \beta_1 & \end{bmatrix}, \quad B_2 = \begin{bmatrix} & 0 & \\ & -\beta_2 & \\ & 0 & \\ \phi & 0 & \phi \\ & 0 & \\ & 0 & \\ & \beta_2 & \end{bmatrix},$$

$$B_3 = \begin{bmatrix} & 0 & \\ & 0 & \\ & -\beta_3 & \\ \phi & 0 & \phi \\ & 0 & \\ & 0 & \\ & \beta_3 & \end{bmatrix}, \quad B_4 = \begin{bmatrix} & 0 & \\ & 0 & \\ & 0 & \\ \phi & -\beta_4 & \phi \\ & 0 & \\ & 0 & \\ & \beta_4 & \end{bmatrix},$$

$$B_5 = \begin{bmatrix} & 0 & \\ & 0 & \\ & 0 & \\ \phi & 0 & \phi \\ & -\beta_5 & \\ & 0 & \\ & \beta_5 & \end{bmatrix}, \quad B_6 = \begin{bmatrix} & 0 & \\ & 0 & \\ & 0 & \\ \phi & 0 & \phi \\ & 0 & \\ & -\beta_6 & \\ & \beta_6 & \end{bmatrix}$$

$$B_7 = \begin{bmatrix} & b_{17} & \\ & b_{27} & \\ & b_{37} & \\ \phi & b_{47} & \\ & b_{57} & \\ & b_{67} & \\ & -\beta_7 & \end{bmatrix}. \qquad \ldots(3.38)$$

$$b_{i7} = \frac{a_{i7}}{\alpha_7}\beta_7, \quad i = 1,\ldots,6 \qquad \ldots(3.39)$$

Various results above can be specialized to (3.34). Stability of (3.35) depends on the eigenvalues of A and that of (3.36) on the eigenvalues of the system matrix obtained by using the equations for variances and covariance from the lower triangle of the P matrix.

Equation (3.36) can also be expressed in the vector form using the vech operator (see Appendix 5, section A5.2.3.1).

$$\text{vec } \dot{P} = [(I \otimes A) + (A \otimes I)]\text{vec } P + \sum_{i=1}^{7}(B_i \otimes B_i)\text{vec } P$$

$$= [(A \oplus A) + \sum_{i=1}^{7}(B_i \otimes B_i)]\text{vec } P \ ,$$

where $(A \oplus A) = (A \otimes I) + (I \otimes A)$.

$$\text{vech } \dot{P} = H[(A \oplus A) + \sum_{i=1}^{7} (B_i \otimes B_i)]K \text{ vech } P \qquad \ldots(3.40)$$

where H and K are as defined in section A5.2.3.1. Checking the stability of (3.36) is equaivalent to checking the stbility of (3.40).

Results of estimation and their analyses are the topic of the next chapter.

CHAPTER 4

RESULTS OF ESTIMATION AND STATISTICAL ANALYSES

4.0 INTRODUCTORY REMARKS

Stochastic models, both discrete-time and continuous-time were presented in the last chapter. In this chapter their parameter estimation will be given and the results analyzed. The proximity of fit with the experimental data will be assessed statistically. In the case of lack of fit further tests will be performed to look into which compartment(s) might be the main cause of this. First the nature and the shortcomings of the available physiological data will be described. Criteria used during parameter optimization and the statistical tests that will be done will be discussed next. After that the discrete-time model will be taken up, estimation results tabulated, and stability and

statistical tests performed. The same will be done for the continuous-time model.

4.1 THE PHYSIOLOGICAL DATA AVAILABLE.

The Smith & Ford (1983) data was given previously for 13 compartments (section 2.2). It tabulates only means and standard deviations of observations (% activity per organ) at each time point (n) in each organ (i). The sample size (i.e. the number of rats which were sacrificed at each sample time) varies from 2 to 6. Missing data and variations are as follows:

Mean --- not available at (either label present was in trace amounts or was not measureable):

n = 1 for peyer's patches, coeliac LN, right and left popliteal LN,

n = 2 for coeliac LN, right and left popliteal LN, and

n = 5 for right and left popliteal LN.

Standard deviation --- not available for the above 9 points and also not at n = 2 for peyer's patches.

Covariances --- not available.

Sample size (N) :

2 at n = 2 for peyer's patches,

3 at n = 1 for deep cervical LN,

4 at n = 1 for superficial cervical LN and mesenteric LN,

n = 2 for deep cervical LN,

n = 5 for coeliac LN,

6 at n = 540 for all 13 organs, and

5 at all other observation points.

The 13 instants at which measurements were made are (in minutes): 1, 2, 5, 10, 30, 60, 150, 360, 540, 720, 900, 1080, 1440 and there is the initial condition (i.e, at n = 0).

A limited amount of raw data is available in the form of graphs, but it is very hard to interpret. Points for different rats cannot be identified.

Some of the compartments were lumped to form the 7 compartment model as discussed earlier in section 3.4). The new data is given in Table 2 for means and second moments. Like most biological and social data the observations have been made at unequally spaced intervals of time and there seem to be many outliers in the raw data. The estimation procedures mentioned in sections A5.8.1 and A5.8.2 assume equally spaced data. During estimation, no matter what algorithm is used, the values of the parameters have to be optimized by

minimizing some function of the difference between the experimental data and the corresponding estimated values. The criteria used to do this will be discussed next.

Table 2. SMITH & FORD (1983) DATA FOR THE 7 COMPARTMENT MODEL.

FIRST MOMENTS FROM FORD'S DATA
(% activity per compartment)

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	0.00	0.00	0.00	0.00	0.00	0.00	100.00
1	1.15	2.29	19.20	.23	.16	35.50	41.47
2	1.15	2.29	16.37	.29	.21	40.08	36.59
5	1.36	10.56	19.06	1.52	.96	27.63	38.91
10	1.61	19.51	20.91	3.12	2.30	22.97	29.58
30	1.80	38.93	25.73	8.27	5.17	8.46	11.64
60	2.26	39.41	31.77	13.12	7.60	2.50	3.34
150	2.23	32.31	36.46	14.39	7.82	2.73	4.06
360	1.33	21.32	46.36	16.65	8.76	2.33	3.25
540	1.24	17.90	48.24	18.48	9.13	1.85	3.06
720	2.17	19.14	42.21	22.55	9.86	1.66	2.41
900	.71	14.86	47.47	22.50	9.30	2.02	3.13
1080	1.01	15.48	47.22	23.05	8.92	1.86	2.46
1440	.73	15.48	49.51	21.08	8.13	2.03	3.04

SECOND MOMENTS FROM FORD'S DATA
(% activity per compartment)**2

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	0.00	0.00	0.00	0.00	0.00	0.00	10000.
1	1.37	8.45	376.30	.06	.03	1297.9	1744.2
2	1.37	8.07	275.96	.09	.05	1644.0	1342.1
5	2.09	118.48	402.20	2.81	1.15	808.71	1537.0
10	2.79	417.12	445.15	10.32	5.79	551.34	903.49
30	3.72	1545.2	664.07	70.60	27.40	73.13	144.07
60	5.36	1584.3	1011.6	174.30	58.07	6.61	12.48
150	6.64	1055.6	1330.2	209.66	63.01	7.71	17.26
360	1.91	463.36	2151.7	279.41	78.01	5.47	10.68
540	1.84	334.25	2329.9	356.52	84.99	3.49	9.63
720	6.20	377.50	1785.7	519.33	97.96	2.86	6.12
900	.62	231.19	2258.3	508.74	86.70	4.21	10.64
1080	1.27	243.32	2231.1	537.64	80.06	3.63	6.39
1440	.56	240.24	2454.2	447.66	67.17	4.27	9.66

4.2 MINIMIZING CRITERIA.

The estimates obtained from the models at appropriate time instants were used in the cost functions which were minimized using nonlinear optimization, viz., Powell's technique (Fletcher & Powell, 1963; Powell, 1964). Two cost functions were used, weighted least squares and maximum likelihood. In weighted least squares the variances from the experimental data were used as weights so that in effect a chi-squared criterion was minimized. Expressions for weighted least squares and maximum likelihood (assuming independent Gaussian errors) criteria are given in Appendix 6. The case when lognormal errors are assumed is also discussed there.

Before the estimation procedures are presented there will be a digression to describe the statistical tests that will be performed on the results.

4.3 STATISTICAL ANALYSIS.

Once "optimal" estimates of the parameters have been obtained, tests need to be done to check the validity of the model, a model being considered valid if

its performance in terms of its output is similar to that of the real-world system when similar inputs are applied to both. In other words, if the state of the model is so "close" to the state in the experimental data that the difference between the two can still be attributed to chance then the model is valid. Since the states have been thought of as possessing a stochastic component (section 3.4), the model will be valid if the stochastic process generated by the model is drawn from the same population as the real-world data. This intuitively means that the difference between the two (referred to as "residual error" from now on) should have a more or less symmetrical distribution with zero mean in a range where the errors are very small. As the residual error size increases the distribution is increasingly asymmetric. It is impossible to say with any degree of certitude that the two populations under consideration are the same. What can be decided though, is that the two populations are so similar that there is no significant difference between them in a statistical sense.

The statistical analyses are performed on the residual errors, $\underline{e}_r$, between the experimental data and the estimated states.

$$\underline{e}_r(j) = \underline{x}(j) - \hat{\underline{x}}(j) \ , \qquad \ldots(4.1)$$

where

$\underline{e}_r(j)$ = residual errors at instant j,
the components being $e_{r_i}(j)$,
i = 1,...,7 (%activity).

$\underline{x}(j)$ = measured state at j,
components being $x_i(j)$.

$\hat{\underline{x}}(j)$ = estimated state at j,
components being $\hat{x}_i(j)$.

There are 7 sets of residual errors

$$\{e_{r_i}(j),\ j=1,\ldots,13\}, \quad i = 1,\ldots,7 \ .$$

As just mentioned the problem is to decide if the distributions from which they are drawn are similar enough. Thus the hypothesis to be tested (assuming continuous populations) is:

H_0: $F_1(e_{r_1}) = F_2(e_{r_2}) = \ldots = F_7(e_{r_7})$ (null)

H_1: The populations differ in some way (alternative)

$\ldots(4.2)$

Descriptions of the various tests are given in Appendix 7. Three types of statistical analyses were done: the classical one-way analysis of variance, Link and Wallace analysis of variance and Friedman's two-way analysis of variance. The three are compared in the appendix. Multiple comparisons that were done are also discussed. Terms used in the statistical literature are given in parenthesis in the description of the tests.

Before proceeding further a comment on the robustness of the F-test is in order. The test is considered to be robust for "moderate departures" from normality provided the sample sizes are reasonably large, and for "moderate departures" from the constant variance assumption when the sample sizes are approximately equal. It is sensitive to the assumption of equality of variances for unequal sample sizes (Gibbons, 1985). Recently there have been attempts to quantify such traditionally used statements. Tiku et.al. (1986) show that F-test is asymptotically robust with increasing sample size. For small samples recently it has been shown using Laguerre series approximation of the nonnull distribution that if the underlying distribution is skewed (e.g., lognormal) or is symmetric with very large kurtosis then the variance-ratio test is not robust (Tan, 1982; Tiku, et.al., 1986).

4.4 ESTIMATION OF PARAMETERS IN THE DISCRETE MODEL.

4.4.1 ESTIMATION RESULTS.

Since only means and variances are given in the data, equations (A5.7) and (A5.8) were used as dictated by logic. The first moment equation (A5.7) was used to estimate A. It was solved recursively, taking k=1 and starting n at 0. In the case of the second moment equation (A5.8), the diagonal of the matrix $E[\underline{x}(n)\underline{x}'(n)]$ was used to estimate (C_β, G), covariances not being available. The equation used in estimation is

$$\begin{aligned} P_d(n+1) &= \text{diag}\{E[\underline{x}(n+1)\underline{x}'(n+1)]\} \\ &= \text{diag}\{AE[\underline{x}(n)\underline{x}'(n)]A' + E[\beta(n)\underline{x}(n)\underline{x}'(n)\beta'(n)]\} \\ &\quad + \text{diag}\{CE[\underline{\varepsilon}(n+1)\,\underline{\varepsilon}'(n+1)]C\} \end{aligned}$$

$$P_d(n+1) = A_d P_d(n) + C_d P_e(n+1) \qquad \ldots(4.3)$$

where

$$A_d = \begin{bmatrix} (1-\alpha_1)^2+\beta_1^2 & & & & & & \alpha_{17}^2+b_{17}^2 \\ & (1-\alpha_2)^2+\beta_2^2 & & & & & \alpha_{27}^2+b_{27}^2 \\ & & (1-\alpha_3)^2+\beta_3^2 & & & & \alpha_{37}^2+b_{37}^2 \\ & \phi & \alpha_3^2+\beta_3^2 & (1-\alpha_4)^2+\beta_4^2 & & & \alpha_{47}^2+b_{47}^2 \\ & & & & (1-\alpha_5)^2+\beta_5^2 & & \alpha_{57}^2+b_{57}^2 \\ & & & & & (1-\alpha_6)^2+\beta_6^2 & \alpha_{67}^2+b_{67}^2 \\ \alpha_1^2+\beta_1^2 & \alpha_2^2+\beta_2^2 & 0 & \alpha_4^2+\beta_4^2 & \alpha_5^2+\beta_5^2 & \alpha_6^2+\beta_6^2 & (1-\alpha)_7^2+\beta_7^2 \end{bmatrix},$$

$$C_d = \begin{bmatrix} \sigma_{\varepsilon_1}^2 & & & & & & \\ & \sigma_{\varepsilon_2}^2 & & & & & \\ & & \sigma_{\varepsilon_3}^2 & & & & \\ & & & \sigma_{\varepsilon_4}^2 & & & \\ & \phi & & & \sigma_{\varepsilon_5}^2 & & \\ & & & & & \sigma_{\varepsilon_6}^2 & \\ & & & & & & \sigma_{\varepsilon_7}^2 \end{bmatrix},$$

and $$P_e(n) = \text{diag}\{E[\underline{\varepsilon}(n)\underline{\varepsilon}'(n)]\}$$

Some important covariances, like those between blood and the other compartments, are not available in the experimental data. Others, like those between bone marrow and the other compartments can be safely ignored. Equation (4.3) does not take the important covariances into account.

The estimated values and residual errors are presented in Tables 3 to 6 for the first and second moments as least squares and maximum likelihood estimates depending on which minimizing criterion was used. In all of these 4 tables the columns are the compartments in order and the rows the time instants.

The cost functions Q.(.) mentioned in section 4.2 are minimized. $Q_{\chi^2}(.)$ is a function of the sample variance of the residual errors, so that effectively this is what is minimized to obtain the "best" parameter estimates. For the Gaussian assumption, $Q_{-zLL}(.)$ and $Q_{\chi^2}(.)$ have similar interpretation. Being divided by variance from the data as weights, Q.(.) is a dimensionless ratio. For the first moments min $Q_{\chi^2}(\hat{A}) = 5.73$ and min $Q_{-zLL}(\hat{A}) = 6.39$, while for the second moments min $Q_{\chi^2}(\hat{C}_\beta, \hat{G}) = 16428.22$ and min $Q_{-zLL}(\hat{C}_\beta, \hat{G}) = 15.45$. The estimated parameter values are tabulated in Table 7. The minimized χ^2-values (i.e., 91*(min $Q_{\chi^2}(\hat{.})$), see Appendix 6) are significant which indicates that the residual errors are quite large. In other words the model does not fit the data.

Detailed statistical analyses of the estimated results follow Table 7.

Table 3

THE DISCRETE MODEL
WEIGHTED LEAST SQUARES ESTIMATION
FIRST MOMENTS

ESTIMATED VALUES AT OBSERVATION INSTANTS
(% activity per compartment)

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.1000E+03
1	.4859E+00	.6532E+01	.3520E+01	.3529E+00	.3664E+00	.3913E+02	.6473E+02
2	.7947E+00	.1071E+02	.5792E+01	.5857E+00	.6034E+00	.4941E+02	.5121E+02
5	.1425E+01	.1935E+02	.1059E+02	.1098E+01	.1106E+01	.4603E+02	.3829E+02
10	.2143E+01	.2947E+02	.1644E+02	.1774E+01	.1722E+01	.3372E+02	.2799E+02
30	.3162E+01	.4594E+02	.2794E+02	.3535E+01	.2974E+01	.1137E+02	.9914E+01
60	.2991E+01	.4755E+02	.3338E+02	.5238E+01	.3651E+01	.4900E+01	.4648E+01
150	.1993E+01	.3892E+02	.4000E+02	.9399E+01	.4726E+01	.3384E+01	.3310E+01
360	.1161E+01	.2576E+02	.4647E+02	.1652E+02	.6415E+01	.2522E+01	.2472E+01
540	.9466E+00	.2058E+02	.4775E+02	.2007E+02	.7421E+01	.2220E+01	.2180E+01
720	.8612E+00	.1820E+02	.4766E+02	.2201E+02	.8231E+01	.2095E+01	.2058E+01
900	.8269E+00	.1718E+02	.4715E+02	.2294E+02	.8932E+01	.2045E+01	.2010E+01
1080	.8135E+00	.1675E+02	.4660E+02	.2332E+02	.9564E+01	.2026E+01	.1991E+01
1440	.8053E+00	.1650E+02	.4572E+02	.2337E+02	.1068E+02	.2012E+01	.1977E+01

THE MATRIX OF RESIDUAL ERRORS
(% activity per compartment)

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
1	.6641E+00	-.4242E+01	.1568E+02	-.1229E+00	-.2064E+00	-.3631E+01	-.2326E+02
2	.3553E+00	-.8419E+01	.1058E+02	-.2957E+00	-.3934E+00	-.9329E+01	-.1462E+02
5	-.6519E-01	-.8792E+01	.8470E+01	.4223E+00	-.1455E+00	-.1840E+02	.6170E+00
10	-.5334E+00	-.9961E+01	.4469E+01	.1346E+01	.5776E+00	-.1075E+02	.1593E+01
30	-.1362E+01	-.7011E+01	-.2205E+01	.4735E+01	.2196E+01	-.2906E+01	.1726E+01
60	-.7315E+00	-.8144E+01	-.1613E+01	.7882E+01	.3949E+01	-.2400E+01	-.1308E+01
150	.2373E+00	-.6607E+01	-.3537E+01	.4991E+01	.3094E+01	-.6543E+00	.7500E+00
360	.1691E+00	-.4440E+01	-.1072E+00	.1311E+00	.2345E+01	-.1917E+00	.7778E+00
540	.2934E+00	-.2678E+01	.4894E+00	-.1595E+01	.1709E+01	-.3705E+00	.8803E+00
720	.1309E+01	.9357E+00	-.5445E+01	.5442E+00	.1629E+01	-.4347E+00	.3523E+00
900	-.1169E+00	-.2317E+01	.3191E+00	-.4424E+00	.3679E+00	-.2509E-01	.1120E+01
1080	.1965E+00	-.1274E+01	.6169E+00	-.2728E+00	-.6437E+00	-.1660E+00	.4689E+00
1440	-.7533E-01	-.1019E+01	.3785E+01	-.2291E+01	-.2545E+01	.1796E-01	.1063E+01

Table 4

THE DISCRETE MODEL
-2 LN LIKELIHOOD ESTIMATION
FIRST MOMENTS

ESTIMATED VALUES AT OBSERVATION INSTANTS
(% activity per compartment)

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.1000E+03
1	.4885E+00	.6490E+01	.3501E+01	.3536E+00	.3657E+00	.3884E+02	.6492E+02
2	.7997E+00	.1065E+02	.5768E+01	.5874E+00	.6030E+00	.4916E+02	.5138E+02
5	.1435E+01	.1927E+02	.1055E+02	.1102E+01	.1106E+01	.4594E+02	.3840E+02
10	.2158E+01	.2935E+02	.1639E+02	.1780E+01	.1724E+01	.3370E+02	.2810E+02
30	.3179E+01	.4582E+02	.2790E+02	.3547E+01	.2980E+01	.1141E+02	.9996E+01
60	.2998E+01	.4747E+02	.3337E+02	.5251E+01	.3662E+01	.4923E+01	.4688E+01
150	.1980E+01	.3886E+02	.4002E+02	.9405E+01	.4742E+01	.3391E+01	.3331E+01
360	.1149E+01	.2570E+02	.4652E+02	.1651E+02	.6434E+01	.2523E+01	.2484E+01
540	.9379E+00	.2051E+02	.4781E+02	.2005E+02	.7439E+01	.2221E+01	.2190E+01
720	.8539E+00	.1814E+02	.4772E+02	.2198E+02	.8246E+01	.2095E+01	.2067E+01
900	.8202E+00	.1712E+02	.4722E+02	.2291E+02	.8945E+01	.2045E+01	.2018E+01
1080	.8071E+00	.1669E+02	.4668E+02	.2329E+02	.9574E+01	.2026E+01	.2000E+01
1440	.7991E+00	.1644E+02	.4580E+02	.2334E+02	.1068E+02	.2012E+01	.1986E+01

THE MATRIX OF RESIDUAL ERRORS
(% activity per compartment)

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
1	.6615E+00	-.4200E+01	.1570E+02	-.1236E+00	-.2057E+00	-.3343E+01	-.2345E+02
2	.3503E+00	-.8362E+01	.1060E+02	-.2974E+00	-.3930E+00	-.9085E+01	-.1479E+02
5	-.7499E-01	-.8705E+01	.8506E+01	.4183E+00	-.1458E+00	-.1831E+02	.5055E+00
10	-.5478E+00	-.9841E+01	.4517E+01	.1340E+01	.5764E+00	-.1073E+02	.1479E+01
30	-.1379E+01	-.6890E+01	-.2167E+01	.4723E+01	.2190E+01	-.2951E+01	.1644E+01
60	-.7381E+00	-.8062E+01	-.1604E+01	.7869E+01	.3938E+01	-.2423E+01	-.1348E+01
150	.2505E+00	-.6546E+01	-.3561E+01	.4985E+01	.3078E+01	-.6608E+00	.7295E+00
360	.1810E+00	-.4375E+01	-.1594E+00	.1414E+00	.2326E+01	-.1934E+00	.7656E+00
540	.3021E+00	-.2614E+01	.4272E+00	-.1575E+01	.1691E+01	-.3710E+00	.8704E+00
720	.1316E+01	.9979E+00	-.5513E+01	.5705E+00	.1614E+01	-.4348E+00	.3433E+00
900	-.1102E+00	-.2257E+01	.2480E+00	-.4130E+00	.3549E+00	-.2511E-01	.1112E+01
1080	.2029E+00	-.1215E+01	.5437E+00	-.2424E+00	-.6538E+00	-.1660E+00	.4603E+00
1440	-.6906E-01	-.9621E+00	.3709E+01	-.2261E+01	-.2549E+01	.1779E-01	.1054E+01

Table 5

THE DISCRETE MODEL
WEIGHTED LEAST SQUARES ESTIMATION
SECOND MOMENTS

ESTIMATED VALUES AT OBSERVATION INSTANTS
(% activity per compartment)**2

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.1000E+05
1	.7884E-01	.1425E+02	.9318E+01	.9297E+01	.5041E-01	.5113E+03	.1313E+04
2	.1035E-01	.1592E+02	.1498E+02	.1489E+02	.6188E-01	.5128E+03	.5795E+03
5	.3528E-02	.1738E+02	.3075E+02	.3055E+02	.8543E-01	.4067E+03	.4152E+03
10	.2452E-02	.1868E+02	.5607E+02	.5570E+02	.1214E+00	.2747E+03	.2899E+03
30	.7399E-03	.1818E+02	.1479E+03	.1470E+03	.2483E+00	.6648E+02	.8960E+02
60	.3169E-03	.1384E+02	.2637E+03	.2620E+03	.4237E+00	.1906E+02	.3962E+02
150	.1736E-03	.5812E+01	.5005E+03	.4972E+03	.9378E+00	.9028E+01	.2193E+02
360	.1328E-03	.1947E+01	.7203E+03	.7156E+03	.2128E+01	.6704E+01	.1685E+02
540	.1448E-03	.1815E+01	.7718E+03	.7667E+03	.3148E+01	.7286E+01	.1837E+02
720	.1613E-03	.1991E+01	.7877E+03	.7825E+03	.4169E+01	.8119E+01	.2047E+02
900	.1784E-03	.2206E+01	.7927E+03	.7875E+03	.5192E+01	.8985E+01	.2264E+02
1080	.1957E-03	.2427E+01	.7943E+03	.7891E+03	.6217E+01	.9856E+01	.2483E+02
1440	.2303E-03	.2871E+01	.7952E+03	.7900E+03	.8271E+01	.1160E+02	.2922E+02

THE MATRIX OF RESIDUAL ERRORS
(% activity per compartment)**2

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
1	.1052E+01	-.4847E+02	.3546E+03	-.9362E+01	-.1576E+00	-.7446E+03	-.2031E+04
2	.1248E+01	-.2748E+02	.2501E+03	-.2405E+02	-.8088E-01	.7365E+03	.3637E+03
5	.2072E+01	.8248E+02	.3454E+03	-.5297E+02	.9801E+00	.1667E+03	.9466E+03
10	.2773E+01	.3789E+03	.3378E+03	-.9580E+02	.5549E+01	.1165E+03	.4931E+03
30	.3712E+01	.1509E+04	.3728E+03	-.2181E+03	.2691E+02	-.3451E+02	.2090E+02
60	.5356E+01	.1557E+04	.4883E+03	-.3445E+03	.5722E+02	-.2599E+02	-.3992E+02
150	.6636E+01	.1044E+04	.3326E+03	-.7795E+03	.6114E+02	-.8119E+01	-.1144E+02
360	.1913E+01	.4594E+03	.7138E+03	-.1146E+04	.7376E+02	-.6353E+01	-.1131E+02
540	.1839E+01	.3306E+03	.7889E+03	-.1172E+04	.7870E+02	-.9365E+01	-.1434E+02
720	.6196E+01	.3735E+03	.2129E+03	-.1040E+04	.8963E+02	-.1146E+02	-.2059E+02
900	.6188E+00	.2267E+03	.6754E+03	-.1061E+04	.7633E+02	-.1164E+02	-.1891E+02
1080	.1269E+01	.2384E+03	.6449E+03	-.1035E+04	.6763E+02	-.1376E+02	-.2602E+02
1440	.5543E+00	.2344E+03	.8663E+03	-.1127E+04	.5064E+02	-.1621E+02	-.2847E+02

Table 6

THE DISCRETE MODEL
-2 LN LIKELIHOOD ESTIMATION
SECOND MOMENTS

ESTIMATED VALUES AT OBSERVATION INSTANTS
(% activity per compartment)**2

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.1000E+05
1	.8059E-01	.1422E+02	.9306E+01	.9287E+01	.5076E-01	.5095E+03	.1309E+04
2	.1055E-01	.1588E+02	.1496E+02	.1486E+02	.6226E-01	.5108E+03	.5772E+03
5	.3590E-02	.1733E+02	.3069E+02	.3049E+02	.8583E-01	.4049E+03	.4133E+03
10	.2493E-02	.1863E+02	.5594E+02	.5558E+02	.1219E+00	.2732E+03	.2883E+03
30	.7503E-03	.1810E+02	.1476E+03	.1466E+03	.2488E+00	.6586E+02	.8889E+02
60	.3214E-03	.1377E+02	.2632E+03	.2615E+03	.4241E+00	.1884E+02	.3931E+02
150	.1762E-03	.5775E+01	.4998E+03	.4966E+03	.9383E+00	.8935E+01	.2178E+02
360	.1350E-03	.1933E+01	.7201E+03	.7154E+03	.2128E+01	.6645E+01	.1676E+02
540	.1472E-03	.1802E+01	.7719E+03	.7669E+03	.3148E+01	.7225E+01	.1828E+02
720	.1641E-03	.1978E+01	.7880E+03	.7828E+03	.4169E+01	.8052E+01	.2037E+02
900	.1816E-03	.2192E+01	.7930E+03	.7879E+03	.5193E+01	.8912E+01	.2254E+02
1080	.1991E-03	.2412E+01	.7947E+03	.7895E+03	.6217E+01	.9778E+01	.2472E+02
1440	.2344E-03	.2854E+01	.7956E+03	.7904E+03	.8272E+01	.1151E+02	.2909E+02

THE MATRIX OF RESIDUAL ERRORS
(% activity per compartment)**2

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
1	.1047E+01	-.4789E+02	.3547E+03	-.9352E+01	-.1575E+00	-.7203E+03	-.2061E+04
2	.1246E+01	-.2733E+02	.2501E+03	-.2401E+02	-.8149E-01	.7420E+03	.3632E+03
5	.2072E+01	.8260E+02	.3455E+03	-.5285E+02	.9793E+00	.1700E+03	.9481E+03
10	.2773E+01	.3790E+03	.3380E+03	-.9556E+02	.5548E+01	.1192E+03	.4946E+03
30	.3712E+01	.1509E+04	.3735E+03	-.2175E+03	.2691E+02	-.3343E+02	.2163E+02
60	.5356E+01	.1557E+04	.4894E+03	-.3435E+03	.5722E+02	-.2558E+02	-.3963E+02
150	.6636E+01	.1044E+04	.3339E+03	-.7783E+03	.6114E+02	-.7933E+01	-.1131E+02
360	.1913E+01	.4595E+03	.7142E+03	-.1146E+04	.7376E+02	-.6229E+01	-.1125E+02
540	.1839E+01	.3306E+03	.7886E+03	-.1172E+04	.7870E+02	-.9236E+01	-.1428E+02
720	.6196E+01	.3735E+03	.2123E+03	-.1041E+04	.8963E+02	-.1132E+02	-.2053E+02
900	.6188E+00	.2267E+03	.6747E+03	-.1062E+04	.7632E+02	-.1149E+02	-.1884E+02
1080	.1269E+01	.2384E+03	.6442E+03	-.1036E+04	.6763E+02	-.1359E+02	-.2595E+02
1440	.5542E+00	.2345E+03	.8655E+03	-.1128E+04	.5064E+02	-.1601E+02	-.2839E+02

Table 7

ESTIMATED PARAMETERS VALUES FOR THE DISCRETE MODEL

WLS = Weighted Least Squares estimation
-2LL = -2 Ln Likelihood estimation

Deterministic Parts of Multiplicative Parameters:

	α_1	α_{12}	α_2	α_{23}	α_3
WLS	.11948E-01	.48594E-02	.78494E-02	.65323E-01	.15659E-02
-2LL	.12159E-01	.48854E-02	.78598E-02	.64899E-01	.15616E-02

	α_{13}	α_4	α_{24}	α_5	α_{35}
WLS	.35198E-01	.33771E-02	.34741E-02	.41243E-03	.36640E-02
-2LL	.35008E-01	.33798E-02	.34816E-02	.41566E-03	.36575E-02

	α_6	α_{45}	α_7
WLS	.38460E+00	.39131E+00	.49617E+00
-2LL	.38342E+00	.38843E+00	.49964E+00

Standard Deviations of the Multiplicative Noises:

	σ_{β_1}	σ_{β_2}	σ_{β_3}	σ_{β_4}	σ_{β_5}
WLS	.10245E-02	.99294E+00	.99673E+00	.15762E-03	.10000E+01
-2LL	.10245E-02	.99294E+00	.99674E+00	.15762E-03	.10000E+01

	σ_{β_6}	σ_{β_7}
WLS	.93364E+00	.29113E+00
-2LL	.93364E+00	.29076E+00

Standard Deviations of the Additive Noises:

	σ_{ε_1}	σ_{ε_2}	σ_{ε_3}	σ_{ε_4}	σ_{ε_5}
WLS	.23895E-04	.19190E-05	.22762E+01	.56450E-05	.74743E-01
-2LL	.23895E-04	.19190E-05	.22733E+01	.56450E-05	.74743E-01

	σ_{ε_6}	σ_{ε_7}
WLS	.37201E-06	.23510E+01
-2LL	.37201E-06	.23481E+01

4.4.2 STABILITY ANALYSIS.

WEIGHTED LEAST SQUARES:

First-moments --- A is an irreducible matrix. It is column stochastic (in the sense of Appendix 1) and thus its spectral radius is 1 (see proposition A2.6 in Appendix 2). The seven compartments are not linearly independent because blood is the sum of the other compartments and overall it is a closed system. The subroutine HQR from EISPACK was used to find the eigenvalues and confirm the value of the spectral radius.

Second-moments --- The matrix $(A \otimes A + C_{\rho})$ is a 49×49 sparse matrix and the usual method for finding eigenvalues are not efficient in such case. Ranges of eigenvalues were estimated using Gershgorin's Circle Theorem (Lancaster, 1969) and the fact that the spectral radius of a matrix is always greater than or equal to the largest diagonal element,

$$\rho(A) \geqslant \max_i a_{ii} \quad .$$

It turned out that the spectral radius was greater than 1 (The eigenvalues lie in the interval [-6.27,8.5] and the spectral radius > 1.9992).

-2 Ln LIKELIHOOD:

First-moments --- The spectral radius of A, $\rho(A) = 1$.

Second-moments --- The spectral radius $\rho(A \otimes A + C_\beta) > 1$. (The eigenvalues are in [-6.26,8.50] and $\rho(.) > 1.9992$).

In both cases the implication is of instability and second-order non-stationarity by propositions A5.1 and A5.4. This being so and the minimized χ^2 (See section 4.4.1) being significant at $\alpha = 0.01$, further analyses were done to check into the goodness of fit of the compartments of the model. Since one of the conditions for existence and convergence of moments (Prop A5.6) is the same as the condition for stability which is violated, by proposition A5.6 second moment diverges. There is a possibility of this happening because the variances were estimated by using only the diagonal of

the $E[\underline{x}(n)\underline{x}'(n)]$ matrix, covariances not being available in the data. By doing so the number of equations used in the estimation of the parameters is reduced and in turn the number of constraints on the parameters is also reduced thus leading to 'mis-estimation'. An hypothetical numerical example will now be given to show how this can occur.

4.4.2.1 AN HYPOTHETICAL NUMERICAL EXAMPLE.

For the time being consider only a two compartment system. Let

$$A = \begin{bmatrix} 1-\alpha_1 & \alpha_2 \\ \alpha_1 & 1-\alpha_2 \end{bmatrix}, \qquad \beta = \begin{bmatrix} -\beta_1 & \beta_2 \\ \beta_1 & -\beta_2 \end{bmatrix}$$

Then

$$(A\otimes A+\beta\otimes\beta) = \begin{bmatrix} (1-\alpha_1)^2+\beta_1^2 & (1-\alpha_1)\alpha_2-\beta_1\beta_2 & (1-\alpha_1)\alpha_2-\beta_1\beta_2 & \alpha_2^2+\beta_2^2 \\ \alpha_1(1-\alpha_1)-\beta_1^2 & (1-\alpha_1)(1-\alpha_2)+\beta_1\beta_2 & \alpha_1\alpha_2+\beta_1\beta_2 & \alpha_2(1-\alpha_2)-\beta_2^2 \\ \alpha_1(1-\alpha_1)-\beta_1^2 & \alpha_1\alpha_2+\beta_1\beta_2 & (1-\alpha_1)(1-\alpha_2)+\beta_1\beta_2 & \alpha_2(1-\alpha_2)-\beta_2^2 \\ \alpha_1^2+\beta_1^2 & \alpha_1(1-\alpha_2)-\beta_1\beta_2 & \alpha_1(1-\alpha_2)-\beta_1\beta_2 & (1-\alpha_2)\alpha_2+\beta_2^2 \end{bmatrix}$$

The second-moment matrix is symmetric, so that only its lower triangle is needed to investigate the stability of

the second moment equation. In this case the matrices H and K of section A5.2.3.1 will be

$$H = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}, \qquad K = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}.$$

Then

$$H(A\otimes A+\beta\otimes\beta)K = \begin{bmatrix} (1-\alpha_1)^2+\beta_1^2 & 2(1-\alpha_1)\alpha_2-2\beta_1\beta_2 & \alpha_2^2+\beta_2^2 \\ \alpha_1(1-\alpha_1)-\beta_1^2 & (1-\alpha_1)(1-\alpha_2)+\alpha_1\alpha_2+2\beta_1\beta_2 & \alpha_2(1-\alpha_2)-\beta_2^2 \\ \alpha_1^2+\beta_1^2 & 2\alpha_1(1-\alpha_2)-2\beta_1\beta_2 & (1-\alpha_2)^2+\beta_2^2 \end{bmatrix}$$

For this matrix, the characteristic equation is

$$\lambda^3-(1+\Delta+\Delta^2+b^2)\lambda^2+(\Delta+\Delta^2+\Delta^3+\Delta b^2+b^2)\lambda-(\Delta^3+b^2\Delta)=0, \qquad \ldots(4.4)$$

where $\Delta = \det(A) = 1-(\alpha_1+\alpha_2)$ and $b = (\beta_1+\beta_2)$.

If only the variances are used in estimation then this 3x3 matrix is replaced by the following 2x2 matrix

$$\begin{bmatrix} (1-\alpha_1)^2+\beta_1^2 & \alpha_2^2+\beta_2^2 \\ \alpha_1^2+\beta_1^2 & (1-\alpha_2)^2+\beta_2^2 \end{bmatrix}.$$

The charateristic equation for this new matrix is

$$\lambda^2-(2\Delta+a^2-2\alpha_1\alpha_2+\beta_1^2+\beta_2^2)\lambda+(\Delta^2+2\alpha_1\alpha_2\Delta+(1-2\alpha_1)\beta_2^2+(1-2\alpha_2)\beta_1^2)=0, \quad \ldots(4.5)$$

where $a = (\alpha_1+\alpha_2)$.

Suppose $\alpha_1=0.1$, $\alpha_2=0.2$, $\beta_1=0.3$, and $\beta_2=0.5$. Then for the above 3×3 matrix the characteristic equation (4.4) becomes

$$\lambda^3 - 2.83\lambda^2 + 2.621\lambda - 0.791 = 0,$$

and the eigenvalues are: $\lambda_1 = -0.00416$, $\lambda_2 = 0.00208+i0.786410$, $\lambda_3 = 0.00208 -i0.786410$. $|\lambda_2| = |\lambda_3| = 0.786413$. The spectral radius is within the unit circle.

For the same values of the parameters the 2×2 matrix has the following characteristic equation (4.5)

$$\lambda^2 - 1.79\lambda + 0.784 = 0,$$

and the eigenvalues are: $\lambda_1 = 1.02548$, and $\lambda_2 = 0.7645$. Thus the spectral radius is greater than one.

This example is for a two-compartment system. If all the equations available are not used from the second-moment matrix there is a possibility of finding

parameter values for which the system diverges. In the 7-compartment system if only seven equations (corresponding to the variances available in the data) are used instead of 28, a similar possibility exists. In fact, this is probably what is happening in the stability analysis.

4.4.3 STATISTICAL ANALYSES.

4.4.3.1 FIRST-MOMENTS.

WEIGHTED LEAST SQUARES --- The traditional approach is to assume independent Gaussian errors and use the classical one-way analysis of variance. The results for this are given in Table 8(Ia). The null and alternative hypotheses are given in equation (A7.1).

The F-test gives $F = 4.61$ ($F_{k-1,N-k;\alpha} < F_{6,60;\alpha} = 3.12$, $k = 7$, $N=91$, $\alpha = 0.01$) which is significant, resulting in the rejection of the null hypothesis. To look deeper into which set(s) (if any) of errors might be the cause of this all pairwise comparisons of means were done using Fisher LSD (Least Significant

Difference) test (See Table 8(Ic)). The means for compartments 2 and 6 are significantly different at α = 0.05 from means for compartments 1, 3, 4, and 5.

Next the assumption for independence of all observations was relaxed, and Link and Wallace analysis of variance based on ranges was done as described above. The computed test statistic K = 1.016. This is critical at α = 0.01 ($K_{n,k;\alpha}$ = 0.79, n=13, k=7, α =0.01), so that again the null hypothesis is rejected. Multiple comparisons were done and results are given in table 8(IIb).

After this the assumption of normality was dropped and Friedman's two-way analysis of variance based on ranks was done. The null hypothesis concerns distributions (not means) as in (4.2). $\hat{\chi}^2_R$ = 25.68 was obtained, which is significant ($\chi^2_{k-1;\alpha}$ = 16.81, k = 7, α = 0.01). Thus the errors in all the compartments do not all have the same distributions. Wilcoxon and Wilcox multiple comparison procedure was used to do all pairwise comparisons. It turns out that the distribution of errors in compartment 2 is significantly different from the distributions in compartments 1, 3, 4, 5, and 7 as seen from Table 8(IIIb).

-2 Ln LIKELIHOOD --- The results were similar as for the weighted least squares and given in Table 9.

4.4.3.2 SECOND-MOMENTS.

WEIGHTED LEAST SQUARES --- Again the three types of analyses were done. The traditional ANOVA and multiple comparisons rejected the null hypothesis (A7.1) ($F = 11.48 > F_{k-1,N-k;\alpha}$ ($< F_{6,60;\alpha} = 3.12$, $k = 7$, $N = 91$, $\alpha = 0.01$)) and give three mutually exclusive subsets of compartment error means (Table 10(Ic)):

$$(\bar{x}_{e_2}, \bar{x}_{e_3}) > (\bar{x}_{e_1}, \bar{x}_{e_5}, \bar{x}_{e_6}, \bar{x}_{e_7}) > (\bar{x}_{e_4})$$

Link and Wallace analysis also rejected the null hypothesis ($K = 1.817 > K_{n,k;\alpha} = 0.79$, $n=13$, $k = 7$, $\alpha = 0.01$). Pairwise comparisons yielded the same subsets as before.

Friedman's analysis showed differences in distributions ($\hat{\chi}^2_R = 44.60 > \chi^2_{k-1;\alpha} = 16.81$, $k = 7$, $\alpha = 0.01$). Wilcoxon and Wilcox test gave three subsets of compartments:

$$\{(1,2,3,5),(1,2,5,6,7),(4)\}.$$

These results will be discussed in chapter 5.

-2 Ln LIKELIHOOD --- The results here were similar to the results for weighted least squares (See table 11).

4.4.4 BIOLOGICAL INTERPRETATION OF THESE RESULTS.

It was shown above that for the estimated parameter values the model mean is marginally stable. Stability of the second moment is a function of the squares of the deterministic parts of the parameters and the variances of the parametric noise. Because of the parametric noise the model is a variable structure system (actually structurally unstable system) with random variation in the structure. As the estimated variances of the noises have large values in the model the distribution of recirculating lymphocytes is unstable. Comparing lymphocyte populations in any compartment there is a big difference in the estimated number of cells and the experimental quantity. For the mean, compartment # 2 (spleen) appears to be the main source of error, while for the variance (standard deviation) compartment #s 3 (LT) and 4 (LN-a) may be the

main cause. There will be a more detailed discussion in chapter 5.

Table 8

STATISTICAL ANALYSES
DISCRETE-TIME MODEL; FIRST MOMENTS
WEIGHTED LEAST SQUARES ESTIMATION

I(a): CLASSICAL ANOVA

Source	SS	df	MS	F
Total	2406.73	90		
Between	596.58	6	99.429	4.61**
Within	1810.15	84	21.549	

I(b): COMPARTMENT ERROR MEANS

$\bar{x}_{e_1} = 2.62$
$\bar{x}_{e_2} = -4.92$
$\bar{x}_{e_3} = 2.42$
$\bar{x}_{e_4} = 1.16$
$\bar{x}_{e_5} = 0.92$
$\bar{x}_{e_6} = -3.79$
$\bar{x}_{e_7} = -2.30$
$\bar{x}_{e_\cdot} = -0.93$

I(c): FISHER LSD TEST

$$LSD = \begin{cases} 3.04 & \alpha = 0.05 \\ 4.84 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_4}$	$\bar{x}_{e_6}$	$\bar{x}_{e_1}$	$\bar{x}_{e_3}$	$\bar{x}_{e_5}$	$\bar{x}_{e_2}$
$\bar{x}_{e_3}$	1.27	1.51	2.40	4.72*	6.21**	7.34**
$\bar{x}_{e_4}$		0.24	1.13	3.46*	4.94**	6.08**
$\bar{x}_{e_6}$			0.89	3.22*	4.71*	5.84**
$\bar{x}_{e_1}$				2.32	3.81*	4.95**
$\bar{x}_{e_7}$					1.49	2.62
$\bar{x}_{e_2}$						1.13

II(a): LINK-WALLACE ANOVA

Ranges: $R_1 = 2.026$, $R_2 = 10.897$, $R_3 = 21.125$, $R_4 = 10.173$, $R_5 = 6.494$, $R_6 = 18.418$, $R_7 = 24.853$, $R_{(\bar{x}_{e_i})} = 7.344$, $\sum_i R_i = 93.986$

From tables (n=13, k=7): $K_{n,k;\infty} = \begin{cases} 0.66 & \alpha = 0.05 \\ 0.79 & \alpha = 0.01 \end{cases}$

$$K = \frac{(13)R_{(\bar{x}_{e_i})}}{\sum R_i} = 1.016^{**}$$

II(b): LINK-WALLACE MULTIPLE COMPARISONS

$$D = \frac{K_{n,k;\alpha}\sum R_i}{n} = \begin{cases} 5.711 & \alpha = 0.01 \\ 4.772 & \alpha = 0.05 \end{cases}$$

	$\bar{x}_{e_4}$	$\bar{x}_{e_6}$	$\bar{x}_{e_1}$	$\bar{x}_{e_3}$	$\bar{x}_{e_5}$	$\bar{x}_{e_2}$
$\bar{x}_{e_3}$	1.27	1.51	2.40	4.72	6.21*	7.34*
$\bar{x}_{e_4}$		0.24	1.13	3.46	4.94*	6.08*
$\bar{x}_{e_6}$			0.89	3.22	4.71	5.84*
$\bar{x}_{e_1}$				2.32	3.81	4.95*
$\bar{x}_{e_7}$					1.49	2.62
$\bar{x}_{e_2}$						1.13

III(a): FRIEDMAN TEST

Compartment #	Sum of Ranks
1	59
2	24
3	64
4	58
5	63
6	34
7	62

$\hat{\chi}^2_R = 25.68^{**}$

III(b): WILCOXON & WLICOX TEST

From tables: $D_{ww;\alpha} = \begin{cases} 32.5 & \alpha = 0.05 \\ 38.0 & \alpha = 0.01 \end{cases}$

	5 (63)	7 (62)	1 (59)	4 (58)	6 (34)	2 (24)
3 (64)	1	2	5	6	30	40**
5 (63)		1	4	5	29	39**
7 (62)			3	4	28	38**
1 (59)				1	25	35*
4 (58)					24	34*
6 (34)						10

* indicates significance at $\alpha = 0.05$
** indicates significance at $\alpha = 0.01$

Table 9

STATISTICAL ANALYSES
DISCRETE-TIME MODEL; FIRST MOMENTS
- 2 ln LIKELIHOOD ESTIMATION

I(a): CLASSICAL ANOVA

Source	SS	df	MS	F
Total	2401.64	90		
Between	586.35	6	97.725	4.52**
Within	1815.29	84	21.611	

I(b): COMPARTMENT ERROR MEANS

$\bar{x}_{e_1} = 0.03$
$\bar{x}_{e_2} = -4.85$
$\bar{x}_{e_3} = 2.40$
$\bar{x}_{e_4} = 1.16$
$\bar{x}_{e_5} = 0.91$
$\bar{x}_{e_6} = -3.74$
$\bar{x}_{e_7} = -2.36$
$\bar{x}_{e_\cdot} = -0.92$

I(c): FISHER LSD TEST

$$LSD = \begin{cases} 3.05 & \alpha = 0.05 \\ 4.85 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_1}$	$\bar{x}_{e_7}$	$\bar{x}_{e_6}$	$\bar{x}_{e_2}$
$\bar{x}_{e_3}$	1.24	1.49	2.38	4.76*	6.15**	7.25**
$\bar{x}_{e_4}$		0.25	1.14	3.52*	4.91**	6.01**
$\bar{x}_{e_5}$			0.88	3.27*	4.65*	5.76**
$\bar{x}_{e_1}$				2.38	3.77*	4.88**
$\bar{x}_{e_7}$					1.39	2.49
$\bar{x}_{e_6}$						1.10

II(a): LINK-WALLACE ANOVA

Ranges: $R_1 = 1.399$, $R_2 = 10.839$, $R_3 = 21.213$, $R_4 = 10.13$, $R_5 = 6.487$, $R_6 = 18.328$, $R_7 = 25.094$, $R_{(\bar{x}_i)} = 7.252$, $\sum R_i = 93.490$

From tables (n=13, k=7): $K_{n,k;\alpha} = \begin{cases} 0.66 & \alpha = 0.05 \\ 0.79 & \alpha = 0.01 \end{cases}$

$$K = \frac{(13) R_{(\bar{x}_i)}}{\sum R_i} = 1.008^{**}$$

II(b): LINK-WALLACE MULTIPLE COMPARISONS

$$D = \frac{K_{n,k;\alpha} \sum R_i}{n} = \begin{cases} 4.746 & \alpha = 0.05 \\ 5.681 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_1}$	$\bar{x}_{e_7}$	$\bar{x}_{e_6}$	$\bar{x}_{e_2}$
$\bar{x}_{e_3}$	1.24	1.49	2.38	4.76*	6.15**	7.25**
$\bar{x}_{e_4}$		0.25	1.14	3.52*	4.91**	6.01**
$\bar{x}_{e_5}$			0.88	3.27*	4.65*	5.76**
$\bar{x}_{e_1}$				2.38	3.77*	4.88**
$\bar{x}_{e_7}$					1.39	2.49
$\bar{x}_{e_6}$						1.10

III(a): FRIEDMAN TEST

Compartment #	Sum of Ranks
1	59
2	24
3	64
4	58
5	63
6	34
7	62

$\hat{\chi}^2_R = 25.68^{**}$

III(b): WILCOXON & WLICOX TEST

From tables: $D_{ww;\alpha} = \begin{cases} 32.5 & \alpha = 0.05 \\ 38.0 & \alpha = 0.01 \end{cases}$

	5 (63)	7 (62)	1 (59)	4 (58)	6 (34)	2 (24)
3 (64)	1	2	5	6	30	40**
5 (63)		1	4	5	29	39**
7 (62)			3	4	28	38**
1 (59)				1	25	35*
4 (58)					24	34*
6 (34)						10

* indicates significance at $\alpha = 0.05$
** indicates significance at $\alpha = 0.01$

Table 10

STATISTICAL ANALYSES
DISCRETE-TIME MODEL; SECOND MOMENTS
WEIGHTED LEAST SQUARES ESTIMATION

I(a): CLASSICAL ANOVA

Source	SS	df	MS	F
Total	24605104.63	90		
Between	11060488.70	6	1843414.78	11.43**
Within	13544615.93	84	161245.43	

I(b): COMPARTMENT ERROR MEANS

$\bar{x}_{e_1}$ = 2.71
$\bar{x}_{e_2}$ = 489.11
$\bar{x}_{e_3}$ = 491.06
$\bar{x}_{e_4}$ = -623.48
$\bar{x}_{e_5}$ = 45.25
$\bar{x}_{e_6}$ = 10.59
$\bar{x}_{e_7}$ = - 29.05
$\bar{x}_{e_.}$ = 55.17

I(c) FISHER LSD TEST:

$$LSD = \begin{cases} 263.02 & \alpha = 0.05 \\ 419.22 & \alpha = 0.01 \end{cases}$$

	e_2	e_5	e_6	e_1	e_7	e_4
$\bar{x}_{e_3}$	1.95	445.81**	480.47**	488.35**	520.12**	1114.54**
$\bar{x}_{e_2}$		443.86**	478.52**	486.40**	518.16**	1112.59**
$\bar{x}_{e_5}$			34.66	42.54	74.30	668.73**
$\bar{x}_{e_6}$				7.88	39.65	634.08**
$\bar{x}_{e_1}$					31.76	626.19**
$\bar{x}_{e_7}$						594.43**

II(a) LINK-WALLACE ANOVA:

Ranges: $R_1 = 6.082$, $R_2 = 1605.47$, $R_3 = 635.4$, $R_4 = 1162.638$, $R_5 = 89.711$, $R_6 = 1481.1$, $R_7 = 2977.6$, $R_{(\bar{x}_{i.})} = 1114.545$, $\sum_i R_i = 7976.001$

From tables (n=13, k=7): $K_{n,k;\alpha} = \begin{cases} 0.66 & \alpha = 0.05 \\ 0.79 & \alpha = 0.01 \end{cases}$

$$K = \frac{(13)R_{(\bar{x}_{i.})}}{\sum_i R_i} = 1.817^{**}$$

II(b) LINK-WALLACE MULTIPLE COMPARISONS:

$$D = \frac{K_{n,k;\alpha} \sum R_i}{n} = \begin{cases} 404.935 & \alpha = 0.05 \\ 484.695 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_2}$	$\bar{x}_{e_5}$	$\bar{x}_{e_6}$	$\bar{x}_{e_1}$	$\bar{x}_{e_7}$	$\bar{x}_{e_4}$
$\bar{x}_{e_3}$	1.95	445.81*	480.47*	488.35*	520.12*	1114.54*
$\bar{x}_{e_2}$		443.86*	478.52*	486.40*	518.16*	1112.59*
$\bar{x}_{e_5}$			34.66	42.54	74.30	668.73*
$\bar{x}_{e_6}$				7.88	39.65	634.08*
$\bar{x}_{e_1}$					31.76	626.19*
$\bar{x}_{e_7}$						594.43*

III(a): FRIEDMAN TEST

Compartment #	Sum of Ranks
1	50
2	72
3	82
4	17
5	58
6	44
7	41

$\hat{\chi}^2_R = 45.33^{**}$

III(b): WILCOXON & WLICOX TEST

From tables: $D_{WW;\alpha} = \begin{cases} 32.5 & \alpha = 0.05 \\ 38.0 & \alpha = 0.01 \end{cases}$

	2 (72)	5 (58)	1 (50)	6 (44)	7 (41)	4 (17)
3 (82)	10	24	32	38**	41**	65**
2 (72)		14	22	28	31	55**
5 (58)			8	14	17	41**
1 (50)				6	9	33*
6 (44)					3	27
7 (41)						24

* indicates significance at $\alpha = 0.05$
** indicates significance at $\alpha = 0.01$

Table 11

STATISTICAL ANALYSES
DISCRETE-TIME MODEL; SECOND MOMENTS
-2 ln LIKELIHOOD ESTIMATION

I(a): CLASSICAL ANOVA

Source	SS	df	MS	F
Total	24709644.73	90		
Between	11064369.39	6	1844061.56	11.35**
Within	13645275.34	84	162443.75	

I(b): COMPARTMENT ERROR MEANS

$\bar{x}_{e_1}$ =	2.71
$\bar{x}_{e_2}$ =	489.20
$\bar{x}_{e_3}$ =	491.12
$\bar{x}_{e_4}$ =	-623.54
$\bar{x}_{e_5}$ =	45.25
$\bar{x}_{e_6}$ =	13.54
$\bar{x}_{e_7}$ =	- 31.54
$\bar{x}_{e_.}$ =	55.32

I(c) FISHER LSD TEST:

$$LSD = \begin{cases} 263.99 & \alpha = 0.05 \\ 420.78 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_2}$	$\bar{x}_{e_5}$	$\bar{x}_{e_6}$	$\bar{x}_{e_1}$	$\bar{x}_{e_7}$	$\bar{x}_{e_4}$
$\bar{x}_{e_3}$	1.92	445.87**	477.58**	488.41**	504.17**	1114.67**
$\bar{x}_{e_2}$		443.95**	475.65**	486.49**	502.25**	1112.74**
$\bar{x}_{e_5}$			31.70	42.54	58.30	668.79**
$\bar{x}_{e_6}$				10.83	26.59	637.09**
$\bar{x}_{e_1}$					15.76	626.25**
$\bar{x}_{e_7}$						610.49**

II(a) LINK-WALLACE ANOVA:

Ranges: $R_1 = 6.082$, $R_2 = 1604.89$, $R_3 = 653.2$, $R_4 = 1162.648$, $R_5 = 89.788$, $R_6 = 1462.3$, $R_7 = 3009.1$, $R_{(\bar{x}_i)} = 1114.667$, $\sum_i R_i = 7988.007$

From tables (n=13, k=7): $K_{n,k,\alpha} = \begin{cases} 0.66 & \alpha = 0.05 \\ 0.79 & \alpha = 0.01 \end{cases}$

$$K = \frac{(13) R_{(\bar{x}_i)}}{\sum R_i} = 1.814^{**}$$

II(b) LINK-WALLACE MULTIPLE COMPARISONS:

$$D = \frac{K_{n,k,\alpha} \sum R_i}{n} = \begin{cases} 405.545 & \alpha = 0.05 \\ 485.425 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_2}$	$\bar{x}_{e_5}$	$\bar{x}_{e_6}$	$\bar{x}_{e_1}$	$\bar{x}_{e_7}$	$\bar{x}_{e_4}$
$\bar{x}_{e_3}$	1.92	445.87*	477.58*	488.41*	504.17*	1114.67*
$\bar{x}_{e_2}$		443.95*	475.65*	486.49*	502.25*	1112.74*
$\bar{x}_{e_5}$			31.70	42.54	58.30	668.79*
$\bar{x}_{e_6}$				10.83	26.59	637.09*
$\bar{x}_{e_1}$					15.76	626.25*
$\bar{x}_{e_7}$						610.49*

III(a): FRIEDMAN TEST

Compartment #	Sum of Ranks
1	50
2	72
3	82
4	17
5	58
6	44
7	41

$\hat{\chi}^2_R = 45.33^{**}$

III(b): WILCOXON & WLICOX TEST

From tables: $D_{ww,\alpha} = \begin{cases} 32.5 & \alpha = 0.05 \\ 38.0 & \alpha = 0.01 \end{cases}$

	3 (82)	2 (72)	5 (58)	1 (50)	6 (44)	7 (41)
2 (72)	10					
5 (58)	24	14				
1 (50)	32	22	8			
6 (44)	38**	28	14	6		
7 (41)	41**	31	17	9	3	
4 (17)	65**	55**	41**	33*	27	24

* indicates significance at $\alpha = 0.05$
** indicates significance at $\alpha = 0.01$

4.5 ESTIMATION OF PARAMETERS IN THE CONTINUOUS-TIME MODEL.

4.5.1 FIRST-MOMENTS.

Since (3.35) is a system of 7 ordinary differential equations, it can be solved numerically using standard procedures. However, it is a stiff system so that instead of the usual fourth-order Runge-Kutta, Treanor's method (Treanor, 1966; Lomax & Bailey, 1967) was used for integration. For better efficiency a variable step-size was used. This in conjunction with Powell's technique mentioned earlier (in section 2.3 and 4.2) was used to optimize the estimates A using (A6.1) and (A6.3). The minimized cost functions were min $Q_{\chi^2}(\hat{A})$ = 3.50 and min $Q_{-2LL}(\hat{A})$ = 5.90. These min Q.(.) values again represent minimized sample variances as before. They are dimensionless because the weights used were the experimental variances which measure deviations in the radiolabel in any compartment as a percentage of the total injected label.

4.5.2 SECOND-MOMENTS.

Equation (3.36) is also a system of ordinary differential equations. Since none of the covariances are available in the experimental data, only the diagonal of the matrix P(t) was used, taking all the off-diagonal elements to be zero (a not wholly correct assumption even for this model). After some algebra (3.36) becomes

$$\dot{P}_d(t) = A_d \; P_d(t) \qquad \ldots(4.6)$$

where $P_d(t) = \text{diag}(P(t))$, and

$$A_d = \begin{bmatrix} (-2\alpha_1 + \beta_1^2) & & & & & & b_{17}^2 \\ & (-2\alpha_2 + \beta_2^2) & & & & & b_{27}^2 \\ & & (-2\alpha_3 + \beta_3^2) & & & & b_{37}^2 \\ & \phi & & (-2\alpha_4 + \beta_4^2) & & & b_{47}^2 \\ & & & & (-2\alpha_5 + \beta_5^2) & & b_{57}^2 \\ & & & & & (-2\alpha_6 + \beta_6^2) & b_{67}^2 \\ \beta_1^2 & \beta_2^2 & 0 & \beta_4^2 & \beta_5^2 & \beta_6^2 & (-2\alpha_7 + \beta_7^2) \end{bmatrix}$$

For α_i in A_d, the estimates $\hat{\alpha}_i$ obtained from (3.35) were used. Again Treanor's method for integration and Powell's technique for optimization with criteria (A6.1) and (A6.3) were used. The minimized cost functions were min $Q_{\chi^2}(\hat{C}_\beta)$ = 40377.94 and min $Q_{-2LL}(\hat{C}_\beta)$ = 16.35. These are dimensionless ratios and can be seen as minimized sample variances.

The estimated values and residual errors for both first and second moments are presented in Tables 12 to 15 as weighted least squares and -2 ln likelihood estimates. In all of these four tables the columns are the compartments in order and the rows the time instants. The estimated parameter values are tabulated in Table 16.

Table 12

THE CONTINUOUS MODEL
WEIGHTED LEAST SQUARES ESTIMATION
FIRST MOMENTS

ESTIMATED VALUES AT OBSERVATION INSTANTS
(% activity per compartment)

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.1000E+03
1	.3322E+00	.3809E+01	.2154E+01	.3350E+00	.3142E+00	.4393E+02	.4913E+02
2	.5808E+00	.6684E+01	.3794E+01	.5927E+00	.5535E+00	.4227E+02	.4552E+02
5	.1220E+01	.1420E+02	.8148E+01	.1290E+01	.1190E+01	.3561E+02	.3834E+02
10	.2004E+01	.2377E+02	.1390E+02	.2252E+01	.2033E+01	.2697E+02	.2907E+02
30	.3156E+01	.4075E+02	.2601E+02	.4649E+01	.3835E+01	.1036E+02	.1124E+02
60	.2934E+01	.4354E+02	.3222E+02	.6639E+01	.4816E+01	.4694E+01	.5159E+01
150	.1762E+01	.3557E+02	.3921E+02	.1083E+02	.6099E+01	.3099E+01	.3427E+01
360	.1019E+01	.2338E+02	.4569E+02	.1730E+02	.7686E+01	.2337E+01	.2586E+01
540	.8656E+00	.1903E+02	.4710E+02	.2019E+02	.8387E+01	.2102E+01	.2326E+01
720	.8104E+00	.1721E+02	.4726E+02	.2164E+02	.8831E+01	.2014E+01	.2229E+01
900	.7910E+00	.1651E+02	.4706E+02	.2229E+02	.9148E+01	.1985E+01	.2197E+01
1080	.7851E+00	.1627E+02	.4682E+02	.2255E+02	.9396E+01	.1977E+01	.2188E+01
1440	.7836E+00	.1618E+02	.4647E+02	.2263E+02	.9764E+01	.1976E+01	.2187E+01

THE MATRIX OF RESIDUAL ERRORS
(% activity per compartment)

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
1	.8178E+00	-.1519E+01	.1705E+02	-.1050E+00	-.1542E+00	-.8430E+01	-.7656E+01
2	.5692E+00	-.4394E+01	.1258E+02	-.3027E+00	-.3435E+00	-.2195E+01	-.8930E+01
5	.1398E+00	-.3638E+01	.1091E+02	.2301E+00	-.2297E+00	-.7984E+01	.5699E+00
10	-.3944E+00	-.4257E+01	.7008E+01	.8678E+00	.2665E+00	-.4002E+01	.5107E+00
30	-.1356E+01	-.1817E+01	-.2815E+00	.3621E+01	.1335E+01	-.1898E+01	.3963E+00
60	-.6741E+00	-.4129E+01	-.4479E+00	.6481E+01	.2784E+01	-.2194E+01	-.1819E+01
150	.4684E+00	-.3262E+01	-.2748E+01	.3556E+01	.1721E+01	-.3690E+00	.6328E+00
360	.3113E+00	-.2065E+01	.6717E+00	-.6482E+00	.1074E+01	-.7419E-02	.6636E+00
540	.3744E+00	-.1125E+01	.1142E+01	-.1713E+01	.7426E+00	-.2519E+00	.7337E+00
720	.1360E+01	.1927E+01	-.5049E+01	.9129E+00	.1029E+01	-.3541E+00	.1806E+00
900	-.8103E-01	-.1654E+01	.4057E+00	.2075E+00	.1516E+00	.3522E-01	.9329E+00
1080	.2249E+00	-.7881E+00	.3976E+00	.4968E+00	-.4762E+00	-.1168E+00	.2718E+00
1440	-.5358E-01	-.6962E+00	.3041E+01	-.1553E+01	-.1634E+01	.5432E-01	.8530E+00

Table 13

THE CONTINUOUS MODEL
-2 LN LIKELIHOOD ESTIMATION
FIRST MOMENTS

ESTIMATED VALUES AT OBSERVATION INSTANTS
(% activity per compartment)

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.1000E+03
1	.3286E+00	.3751E+01	.2144E+01	.3350E+00	.3131E+00	.4412E+02	.4901E+02
2	.5737E+00	.6574E+01	.3771E+01	.5920E+00	.5510E+00	.4254E+02	.4540E+02
5	.1205E+01	.1397E+02	.8096E+01	.1288E+01	.1184E+01	.3593E+02	.3833E+02
10	.1983E+01	.2342E+02	.1383E+02	.2251E+01	.2026E+01	.2732E+02	.2917E+02
30	.3140E+01	.4042E+02	.2599E+02	.4657E+01	.3838E+01	.1058E+02	.1138E+02
60	.2928E+01	.4344E+02	.3224E+02	.6647E+01	.4826E+01	.4749E+01	.5171E+01
150	.1751E+01	.3569E+02	.3916E+02	.1080E+02	.6098E+01	.3100E+01	.3398E+01
360	.1009E+01	.2357E+02	.4560E+02	.1723E+02	.7677E+01	.2346E+01	.2572E+01
540	.8569E+00	.1916E+02	.4704E+02	.2013E+02	.8382E+01	.2109E+01	.2314E+01
720	.8016E+00	.1730E+02	.4723E+02	.2160E+02	.8830E+01	.2019E+01	.2215E+01
900	.7817E+00	.1657E+02	.4705E+02	.2227E+02	.9151E+01	.1989E+01	.2182E+01
1080	.7754E+00	.1630E+02	.4681E+02	.2255E+02	.9402E+01	.1980E+01	.2172E+01
1440	.7736E+00	.1619E+02	.4646E+02	.2264E+02	.9774E+01	.1978E+01	.2170E+01

THE MATRIX OF RESIDUAL ERRORS
(% activity per compartment)

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
1	.8214E+00	-.1461E+01	.1706E+02	-.1050E+00	-.1531E+00	-.8620E+01	-.7539E+01
2	.5763E+00	-.4284E+01	.1260E+02	-.3020E+00	-.3410E+00	-.2461E+01	-.8807E+01
5	.1547E+00	-.3406E+01	.1096E+02	.2321E+00	-.2240E+00	-.8300E+01	.5787E+00
10	-.3730E+00	-.3911E+01	.7079E+01	.8695E+00	.2737E+00	-.4346E+01	.4067E+00
30	-.1340E+01	-.1495E+01	-.2580E+00	.3613E+01	.1332E+01	-.2117E+01	.2641E+00
60	-.6682E+00	-.4027E+01	-.4711E+00	.6473E+01	.2774E+01	-.2249E+01	-.1831E+01
150	.4787E+00	-.3379E+01	-.2699E+01	.3586E+01	.1722E+01	-.3703E+00	.6619E+00
360	.3213E+00	-.2251E+01	.7613E+00	-.5762E+00	.1083E+01	-.1577E-01	.6775E+00
540	.3831E+00	-.1264E+01	.1198E+01	-.1650E+01	.7478E+00	-.2593E+00	.7463E+00
720	.1368E+01	.1840E+01	-.5022E+01	.9541E+00	.1030E+01	-.3594E+00	.1947E+00
900	-.7171E-01	-.1705E+01	.4183E+00	.2282E+00	.1486E+00	.3140E-01	.9484E+00
1080	.2346E+00	-.8195E+00	.4053E+00	.5030E+00	-.4818E+00	-.1197E+00	.2880E+00
1440	-.4360E-01	-.7135E+00	.3051E+01	-.1559E+01	-.1644E+01	.5187E-01	.8698E+00

Table 14

THE CONTINUOUS MODEL
WEIGHTED LEAST SQUARES ESTIMATION
SECOND MOMENTS

ESTIMATED VALUES AT OBSERVATION INSTANTS
(% activity per compartment)**2

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.1000E+05
1	.1468E+00	.1918E+02	.6099E+01	.1703E+00	.1296E+00	.3132E+04	.3888E+04
2	.2037E+00	.2667E+02	.8494E+01	.2789E+00	.1806E+00	.1269E+04	.1512E+04
5	.2372E+00	.3126E+02	.1002E+02	.5092E+00	.2134E+00	.7501E+02	.8936E+02
10	.2381E+00	.3177E+02	.1031E+02	.8505E+00	.2201E+00	.1063E+01	.1407E+01
30	.2334E+00	.3271E+02	.1115E+02	.2153E+01	.2404E+00	.4129E+00	.6373E+00
60	.2266E+00	.3418E+02	.1254E+02	.3976E+01	.2743E+00	.4311E+00	.6654E+00
150	.2075E+00	.3898E+02	.1782E+02	.9060E+01	.4074E+00	.4911E+00	.7580E+00
360	.1701E+00	.5297E+02	.4035E+02	.2429E+02	.1022E+01	.6671E+00	.1030E+01
540	.1451E+00	.6889E+02	.8113E+02	.4950E+02	.2243E+01	.8702E+00	.1343E+01
720	.1257E+00	.8961E+02	.1629E+03	.9960E+02	.4919E+01	.1139E+01	.1759E+01
900	.1115E+00	.1166E+03	.3269E+03	.2000E+03	.1078E+02	.1500E+01	.2316E+01
1080	.1023E+00	.1517E+03	.6556E+03	.4011E+03	.2360E+02	.1994E+01	.3077E+01
1440	.9926E-01	.2570E+03	.2635E+04	.1612E+04	.1132E+03	.3699E+01	.5710E+01

THE MATRIX OF RESIDUAL ERRORS
(% activity per compartment)**2

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
1	.1220E+01	-.1074E+02	.3702E+03	-.1143E+00	-.1026E+00	-.1834E+04	-.2144E+04
2	.1163E+01	-.1860E+02	.2675E+03	-.1877E+00	-.1316E+00	.3755E+03	-.1696E+03
5	.1853E+01	.8722E+02	.3922E+03	.2303E+01	.9359E+00	.7337E+03	.1448E+04
10	.2548E+01	.3854E+03	.4348E+03	.9473E+01	.5569E+01	.5503E+03	.9021E+03
30	.3483E+01	.1513E+04	.6529E+03	.6844E+02	.2716E+02	.7272E+02	.1434E+03
60	.5131E+01	.1550E+04	.9990E+03	.1703E+03	.5779E+02	.6179E+01	.1181E+02
150	.6430E+01	.1017E+04	.1312E+04	.2006E+03	.6260E+02	.7222E+01	.1650E+02
360	.1743E+01	.4104E+03	.2111E+04	.2551E+03	.7698E+02	.4802E+01	.9648E+01
540	.1695E+01	.2654E+03	.2249E+04	.3070E+03	.8275E+02	.2620E+01	.8291E+01
720	.6072E+01	.2879E+03	.1623E+04	.4197E+03	.9304E+02	.1725E+01	.4363E+01
900	.5082E+00	.1146E+03	.1931E+04	.3088E+03	.7592E+02	.2710E+01	.8327E+01
1080	.1168E+01	.9166E+02	.1575E+04	.1365E+03	.5645E+02	.1634E+01	.3311E+01
1440	.4561E+00	-.1676E+02	-.1806E+03	-.1165E+04	-.4598E+02	.5661E+00	.3954E+01

Table 15

THE CONTINUOUS MODEL
-2 LN LIKELIHOOD ESTIMATION
SECOND MOMENTS

ESTIMATED VALUES AT OBSERVATION INSTANTS
(% activity per compartment)**2

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
0	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.0000E+00	.1000E+05
1	.1447E+00	.1872E+02	.6079E+01	.1711E+00	.1296E+00	.3160E+04	.3891E+04
2	.2011E+00	.2603E+02	.8467E+01	.2797E+00	.1806E+00	.1285E+04	.1514E+04
5	.2354E+00	.3052E+02	.9993E+01	.5087E+00	.2135E+00	.7611E+02	.8962E+02
10	.2386E+00	.3102E+02	.1028E+02	.8473E+00	.2201E+00	.1061E+01	.1386E+01
30	.2431E+00	.3195E+02	.1112E+02	.2141E+01	.2403E+00	.3982E+00	.6108E+00
60	.2499E+00	.3340E+02	.1250E+02	.3955E+01	.2741E+00	.4163E+00	.6386E+00
150	.2715E+00	.3816E+02	.1776E+02	.9031E+01	.4066E+00	.4757E+00	.7297E+00
360	.3300E+00	.5206E+02	.4022E+02	.2427E+02	.1017E+01	.6502E+00	.9973E+00
540	.3905E+00	.6795E+02	.8087E+02	.4946E+02	.2226E+01	.8517E+00	.1306E+01
720	.4627E+00	.8869E+02	.1624E+03	.9954E+02	.4869E+01	.1119E+01	.1717E+01
900	.5492E+00	.1158E+03	.3259E+03	.1998E+03	.1064E+02	.1479E+01	.2268E+01
1080	.6531E+00	.1511E+03	.6536E+03	.4009E+03	.2325E+02	.1970E+01	.3023E+01
1440	.9302E+00	.2579E+03	.2627E+04	.1612E+04	.1109E+03	.3672E+01	.5633E+01

THE MATRIX OF RESIDUAL ERRORS
(% activity per compartment)**2

COMPARTMENT NUMBER

Time	#1	#2	#3	#4	#5	#6	#7
1	.1222E+01	-.1027E+02	.3702E+03	-.1151E+00	-.1026E+00	-.1862E+04	-.2146E+04
2	.1165E+01	-.1796E+02	.2675E+03	-.1885E+00	-.1316E+00	.3594E+03	-.1715E+03
5	.1854E+01	.8796E+02	.3922E+03	.2304E+01	.9358E+00	.7326E+03	.1447E+04
10	.2547E+01	.3861E+03	.4349E+03	.9476E+01	.5569E+01	.5503E+03	.9021E+03
30	.3473E+01	.1513E+04	.6530E+03	.6845E+02	.2716E+02	.7274E+02	.1435E+03
60	.5108E+01	.1551E+04	.9991E+03	.1703E+03	.5779E+02	.6194E+01	.1184E+02
150	.6365E+01	.1017E+04	.1312E+04	.2006E+03	.6260E+02	.7237E+01	.1653E+02
360	.1583E+01	.4113E+03	.2111E+04	.2551E+03	.7699E+02	.4819E+01	.9681E+01
540	.1450E+01	.2663E+03	.2249E+04	.3071E+03	.8277E+02	.2638E+01	.8328E+01
720	.5735E+01	.2888E+03	.1623E+04	.4198E+03	.9309E+02	.1745E+01	.4405E+01
900	.7047E-01	.1154E+03	.1932E+04	.3089E+03	.7606E+02	.2731E+01	.8375E+01
1080	.6170E+00	.9218E+02	.1577E+04	.1367E+03	.5680E+02	.1657E+01	.3365E+01
1440	-.3748E+00	-.1763E+02	-.1728E+03	-.1164E+04	-.4375E+02	.5931E+00	.4031E+01

Table 16

ESTIMATED PARAMETERS VALUES FOR THE CONTINUOUS MODEL

WLS = Weighted Least Squares estimation
-2LL = -2 Ln Likelihood estimation

Deterministic Parts of Multiplicative Parameters:

	α_1	α_{17}	α_2	α_{27}	α_3
WLS	.15153E-01	.54293E-02	.83882E-02	.62028E-01	.16602E-02
-2LL	.15061E-01	.53688E-02	.81857E-02	.61058E-01	.16398E-02

	α_{37}	α_4	α_{47}	α_5	α_{57}
WLS	.34949E-01	.39357E-02	.54088E-02	.10552E-02	.50949E-02
-2LL	.34769E-01	.38872E-02	.54093E-02	.10401E-02	.50769E-02

	α_6	α_{67}	α_7
WLS	.20624E+01	.18631E+01	.19760E+01
-2LL	.20247E+01	.18455E+01	.19572E+01

Standard Deviations of the Multiplicative Noises:

	σ_{β_1}	σ_{β_2}	σ_{β_3}	σ_{β_4}	σ_{β_5}
WLS	.17103E+00	.13483E+00	.84741E-01	.90577E-04	.80389E-01
-2LL	.17603E+00	.13340E+00	.84503E-01	.12462E-03	.80116E-01

	σ_{β_6}	σ_{β_7}
WLS	.86367E-02	.17341E+01
-2LL	.58245E-02	.17234E+01

4.5.3 STABILITY.

WEIGHTED LEAST SQUARES:

First-moment --- A is a compartmental matrix whose column sums are zero and thus its spectral radius is zero (Propositions A2.5 and A2.6 and the system being closed). Subroutine HQR from EISPACK yielded the same result.

Second-moment --- Since B have not been estimated from complete information that could have been available to obtain better estimates, it was not thought worthwhile to find the stability of the second moments (particularly when the Q-values were so high as seen in section 4.5.1). As for the discrete model instead of 28 equations only 7 were used in estimation for the same reason as before. One would expect problems similar to the ones in the variance estimation in the discrete case to arise here also.

-2 Ln LIKELIHOOD:

First-moment --- Spectral radius of A, $\rho(A) = 0$

Second-moment --- as for Weighted Least Squares above.

The minimized $Q_{\chi^2}(C_\beta)$ = 40377.94 (as in sections 4.5.2) is significant at α = 0.01. Further analyses were done to check into the goodness of fit of the model. These are discussed in the next section.

4.5.4 STATISTICAL ANALYSES.

4.5.4.1 FIRST-MOMENTS.

WEIGHTED LEAST SQUARES --- The traditional approach is to assume independent Gaussian errors and do the classical one-way analysis of variance. The results for this are given in table 17(Ia). The null and alternative hypotheses are the same as (A7.1). The F-test gives F = 4.77 ($F_{k-1,N-k;\alpha} < F_{6,60;\alpha}$ =3.12, k = 7, N=91, α = 0.01) which is significant, resulting in the rejection of the null hypothesis (A7.1). To look deeper

into which set(s) (if any) of errors might be the cause of this all pairwise comparisons of means were done using Fisher LSD (Least Significant Difference) test (See table 17(Ic)). There are three mutually exclusive subsets with significantly different (at $\alpha = 0.05$) means

$$(\bar{x}_{e_3}) > (\bar{x}_{e_1}, \bar{x}_{e_4}, \bar{x}_{e_5}, \bar{x}_{e_7}) > (\bar{x}_{e_2}, \bar{x}_{e_6}).$$

Just as for the discrete case, next the assumption for independence was relaxed and Link and Wallace analysis of variance based on ranges was done. The test statistic K = 1.166. This is critical at $\alpha = 0.01$ ($K_{n,k;\alpha} = 0.79$, n=13, k=7), so that again the null hypothesis (A7.1) is rejected. Results of multiple comparisons are in table 17(IIb).

After this the assumption of normality was eliminated and Friedman's two-way analysis of variance based on ranks was done and hypothesis (4.2) tested. $\hat{\chi}^2_R = 26.74$ was obtained, which is significant ($\chi^2_{k-1;\alpha} = 16.81$, k = 7, $\alpha = 0.01$). Thus the errors in all the compartments do not all have the same distributions. Wilcoxon and Wilcox multiple comparison procedure was used to do all pairwise comparisons. It turns out that the distribution of errors in compartments 2 and 3 are

significantly different from one another, but in almost all compartments though the distributions are different they are "quite close". (See Table 17(IIIb))

-2 Ln LIKELIHOOD --- The results were similar and are given in table 18.

4.5.4.2 SECOND-MOMENTS.

WEIGHTED LEAST SQUARES --- Again the three types of analyses were done. The traditional ANOVA and multiple comparisons rejected the null hypothesis (A7.1) ($F = 6.81 > F_{k-1,N-k;\alpha} < F_{6,60;\alpha} = 3.12$, $k = 7$, $N = 91$, $\alpha = 0.01$) and give three mutually exclusive subsets of compartment error means:

$$(\bar{x}_{e_3}) > (\bar{x}_{e_2}) > (\bar{x}_{e_1}, \bar{x}_{e_4}, \bar{x}_{e_5}, \bar{x}_{e_6}, \bar{x}_{e_7})$$

Link and Wallace analysis also rejected the (A7.1) null hypothesis ($K = 1.166 > K_{n,k;\alpha} = 0.79$, $n=13$, $k = 7$, $\alpha = 0.01$). Pairwise comparisons yielded two mutually subsets:.

$$(\bar{x}_{e_3}) > (\bar{x}_{e_1}, \bar{x}_{e_2}, \bar{x}_{e_4}, \bar{x}_{e_5}, \bar{x}_{e_6}, \bar{x}_{e_7}).$$

Friedman's analysis showed differences in distributions ($\hat{\chi}^2_R = 25.12 > \chi^2_{k-1;\alpha} = 16.81$, $k = 7$, $\alpha = 0.01$). Wilcoxon and Wilcox test showed differences in distributions without giving any mutually exclusive subsets. (See table 19)

-2 Ln LIKELIHOOD --- The results here were similar to those above (See table 20).

4.5.5 BIOLOGICAL INTERPRETATION OF THESE RESULTS.

This model is also unstable because of the estimated values of the variances. For the mean value, the main source of error lies in compartments 2 and 3, while for the variance it is in compartments 1 and 3. More discussion will follow in the next chapter.

Table 17

STATISTICAL ANALYSES
CONTINUOUS-TIME MODEL; FIRST MOMENTS
WEIGHTED LEAST SQUARES ESTIMATION

I(a): CLASSICAL ANOVA

Source	SS	df	MS	F
Total	1168.42	90		
Between	297.10	6	49.52	4.77**
Within	871.32	84	10.37	

I(b): COMPARTMENT ERROR MEANS

$\bar{x}_{e_1} = 0.13$
$\bar{x}_{e_2} = -2.11$
$\bar{x}_{e_3} = 3.44$
$\bar{x}_{e_4} = 0.93$
$\bar{x}_{e_5} = 0.48$
$\bar{x}_{e_6} = -2.13$
$\bar{x}_{e_7} = -0.97$
$\bar{x}_{e_8} = -3.39$

I(c): FISHER LSD TEST

$$LSD = \begin{cases} 2.11 & \alpha = 0.05 \\ 3.36 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_1}$	$\bar{x}_{e_7}$	$\bar{x}_{e_2}$	$\bar{x}_{e_6}$
$\bar{x}_{e_3}$	2.51*	2.95*	3.31*	4.41**	5.55**	5.57**
$\bar{x}_{e_4}$		0.45	0.80	1.90	3.04*	3.06*
$\bar{x}_{e_5}$			0.35	1.45	2.59*	2.61*
$\bar{x}_{e_1}$				1.11	2.24*	2.26*
$\bar{x}_{e_7}$					1.14	1.16
$\bar{x}_{e_2}$						0.02

II(a): LINK-WALLACE ANOVA

Ranges: $R_1 = 2.716$, $R_2 = 6.321$, $R_3 = 22.099$, $R_4 = 8.194$, $R_5 = 4.418$, $R_6 = 8.484$, $R_7 = 9.863$, $R_{(\bar{x}_i)} = 5.569$, $\sum_i R_i = 62.095$

From tables (n=13, k=7): $K_{n,k;\alpha} = \begin{cases} 0.66 & \alpha = 0.05 \\ 0.79 & \alpha = 0.01 \end{cases}$

$$K = \frac{(13)R_{(\bar{x}_i)}}{\sum R_i} = 1.169^{**}$$

II(b): LINK-WALLACE MULTIPLE COMPARISONS

$$D = \frac{K_{n,k;\alpha} \sum R_i}{n} = \begin{cases} 3.153 & \alpha = 0.05 \\ 3.773 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_1}$	$\bar{x}_{e_3}$	$\bar{x}_{e_2}$	$\bar{x}_{e_6}$
$\bar{x}_{e_3}$	2.51*	2.95*	3.31*	4.41**	5.55**	5.57**
$\bar{x}_{e_4}$		0.45	0.80	1.90	3.04*	3.06*
$\bar{x}_{e_5}$			0.35	1.45	2.59*	2.61*
$\bar{x}_{e_1}$				1.11	2.24*	2.26*
$\bar{x}_{e_7}$					1.14	1.16
$\bar{x}_{e_2}$						0.02

III(a): FRIEDMAN TEST

Compartment #	Sum of Ranks
1	54
2	27
3	72
4	63
5	58
6	32
7	58

3 (72)
4 (63)
5 (58)
7 (58)
1 (54)
6 (32)

$\hat{\chi}^2_R = 26.74^{**}$

III(b): WILCOXON & WLICOX TEST

From tables: $D_{WW;\alpha} = \begin{cases} 32.5 & \alpha = 0.05 \\ 38.0 & \alpha = 0.01 \end{cases}$

4 (63)	5 (58)	7 (58)	1 (54)	6 (32)	2 (27)
9	14	14	18	40**	45**
	5	5	9	31	36*
		0	4	26	31
			4	26	31
				22	27
					5

* indicates significance at $\alpha = 0.05$
** indicates significance at $\alpha = 0.01$

Table 18

STATISTICAL ANALYSES
CONTINUOUS-TIME MODEL; FIRST MOMENTS
-2 ln LIKELIHOOD ESTIMATION

I(a): CLASSICAL ANOVA

Source	SS	df	MS	F
Total	1175.06	90		
Between	304.05	6	50.67	4.89**
Within	871.01	84	10.37	

I(b): COMPARTMENT ERROR MEANS

$\bar{x}_{e_1}$ = 0.14
$\bar{x}_{e_2}$ = -2.07
$\bar{x}_{e_3}$ = 3.47
$\bar{x}_{e_4}$ = 0.94
$\bar{x}_{e_5}$ = 0.48
$\bar{x}_{e_6}$ = -2.24
$\bar{x}_{e_7}$ = -0.96
$\bar{x}_{e}$ = -3.40E-02

I(c): FISHER LSD TEST

$$LSD = \begin{cases} 2.11 & \alpha = 0.05 \\ 3.36 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_1}$	$\bar{x}_{e_7}$	$\bar{x}_{e_2}$	$\bar{x}_{e_6}$
$\bar{x}_{e_3}$	2.52*	2.99*	3.33*	4.43**	5.54**	5.71**
$\bar{x}_{e_4}$		0.46	0.80	1.91	3.01*	3.18*
$\bar{x}_{e_5}$			0.34	1.45	2.55*	2.72*
$\bar{x}_{e_1}$				1.11	2.21*	2.38*
$\bar{x}_{e_7}$					1.10	1.28
$\bar{x}_{e_2}$						0.17

II(a): LINK-WALLACE ANOVA

Ranges: $R_1 = 2.708$, $R_2 = 6.124$, $R_3 = 22.082$, $R_4 = 8.123$, $R_5 = 4.418$, $R_6 = 8.672$, $R_7 = 9.755$, $R_{(\bar{x}_i)} = 5.709$, $\sum_i R_i = 61.882$

From tables (n=13, k=7): $K_{n,k;\alpha} = \begin{cases} 0.66 & \alpha = 0.05 \\ 0.79 & \alpha = 0.01 \end{cases}$

$$K = \frac{(13) R_{(\bar{x}_i)}}{\sum R_i} = 1.199^{**}$$

II(b): LINK-WALLACE MULTIPLE COMPARISONS

$$D = \frac{K_{n,k;\alpha} \sum R_i}{n} = \begin{cases} 3.142 & \alpha = 0.05 \\ 3.761 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_1}$	$\bar{x}_{e_7}$	$\bar{x}_{e_2}$	$\bar{x}_{e_6}$
$\bar{x}_{e_3}$	2.52	2.99	3.33*	4.43**	5.54**	5.71**
$\bar{x}_{e_4}$		0.46	0.80	1.91	3.01*	3.18*
$\bar{x}_{e_5}$			0.34	1.45	2.55*	2.72*
$\bar{x}_{e_1}$				1.11	2.21*	2.38*
$\bar{x}_{e_7}$					1.10	1.28
$\bar{x}_{e_2}$						0.17

III(a): FRIEDMAN TEST

Compartment #	Sum of Ranks
1	54
2	28
3	72
4	63
5	58
6	31
7	58

3 (72)
4 (63)
5 (58)
7 (58)
1 (54)
6 (31)

$\hat{\chi}^2_R = 26.74^{**}$

III(b): WILCOXON & WLICOX TEST

From tables: $D_{ww;\alpha} = \begin{cases} 32.5 & \alpha = 0.05 \\ 38.0 & \alpha = 0.01 \end{cases}$

	4 (63)	5 (58)	7 (58)	1 (54)	6 (31)	2 (28)
3 (72)	9	14	14	18	41**	44**
4 (63)		5	5	9	32	35*
5 (58)			0	4	27	30
7 (58)				4	27	30
1 (54)					23	26
6 (31)						4

* indicates significance at $\alpha = 0.05$
** indicates significance at $\alpha = 0.01$

Table 19

STATISTICAL ANALYSES
CONTINUOUS-TIME MODEL; SECOND MOMENTS
WEIGHTED LEAST SQUARES ESTIMATION

I(a): CLASSICAL ANOVA

Source	SS	df	MS	F
Total	37372983.54	90		
Between	12291293.97	6	2048548.99	6.86**
Within	25081689.57	84	298591.54	

I(b): COMPARTMENT ERROR MEANS

$\bar{x}_{e_1}$ = 2.57
$\bar{x}_{e_2}$ = 436.65
$\bar{x}_{e_3}$ = 1056.69
$\bar{x}_{e_4}$ = 54.84
$\bar{x}_{e_5}$ = 37.92
$\bar{x}_{e_6}$ = - 5.71
$\bar{x}_{e_7}$ = - 19.02
$\bar{x}_{e_.}$ = 228.86

I(c): FISHER LSD TEST

$$LSD = \begin{cases} 357.92 & \alpha = 0.05 \\ 570.48 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_2}$	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_7}$	$\bar{x}_{e_1}$	$\bar{x}_{e_6}$
$\bar{x}_{e_3}$	620.04**	1001.85**	1018.77**	1037.67**	1054.12**	1062.40**
$\bar{x}_{e_2}$		381.81*	398.73*	417.63*	434.08*	442.36*
$\bar{x}_{e_4}$			16.92	35.82	52.26	60.55
$\bar{x}_{e_5}$				18.90	35.35	43.63
$\bar{x}_{e_7}$					16.45	24.73
$\bar{x}_{e_1}$						8.28

II(a): LINK-WALLACE ANOVA

Ranges: $R_1 = 5.974$, $R_2 = 1568.6$, $R_3 = 2429.6$, $R_4 = 1584.7$, $R_5 = 139.02$, $R_6 = 2567.7$, $R_7 = 3591.$, $R_{(\bar{x}_{i\cdot})} = 1062.40$, $\sum_i R_i = 11886.59$

From tables (n=13, k=7): $K_{n,k;\alpha} = \begin{cases} 0.66 & \alpha = 0.05 \\ 0.79 & \alpha = 0.01 \end{cases}$

$$K = \frac{(13) R_{(\bar{x}_{i\cdot})}}{\sum R_i} = 1.162^{**}$$

II(b): LINK-WALLACE MULTIPLE COMPARISONS

$$D = \frac{K_{n,k;\alpha} \sum R_i}{n} = \begin{cases} 603.473 & \alpha = 0.05 \\ 722.939 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_2}$	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_7}$	$\bar{x}_{e_1}$	$\bar{x}_{e_6}$
$\bar{x}_{e_3}$	620.04**	1001.85**	1018.77**	1037.67**	1054.12**	1062.40**
$\bar{x}_{e_2}$		381.81*	398.73*	417.63*	434.08*	442.36*
$\bar{x}_{e_4}$			16.92	35.82	52.26	60.55
$\bar{x}_{e_5}$				18.90	35.35	43.63
$\bar{x}_{e_7}$					16.45	24.73
$\bar{x}_{e_1}$						8.28

III(a): FRIEDMAN TEST

Compartment #	Sum of Ranks
1	29
2	63
3	79
4	56
5	45
6	44
7	48

3 (79)
2 (63)
4 (56)
7 (48)
5 (45)
6 (44)

$\hat{\chi}^2_R = 25.12^{**}$

III(b): WILCOXON & WLICOX TEST

From tables: $D_{ww;\alpha} = \begin{cases} 32.5 & \alpha = 0.05 \\ 38.0 & \alpha = 0.01 \end{cases}$

	2 (63)	4 (56)	7 (48)	5 (45)	6 (44)	1 (29)
3 (79)	16	23	31	34*	35*	50**
2 (63)		7	15	18	19	34*
4 (56)			8	11	12	27
7 (48)				3	4	19
5 (45)					1	16
6 (44)						15

* indicates significance at $\alpha = 0.05$
** indicates significance at $\alpha = 0.01$

Table 20

STATISTICAL ANALYSES
CONTINUOUS-TIME MODEL; SECOND MOMENTS
-2 ln LIKELIHOOD ESTIMATION

I(a): CLASSICAL ANOVA

Source	SS	df	MS	F
Total	37499418.50	90		
Between	12335760.64	6	2055960.14	6.86**
Within	25163657.64	84	299567.35	

I(b): COMPARTMENT ERROR MEANS

$\bar{x}_{e_1}$ = 2.37
$\bar{x}_{e_2}$ = 437.17
$\bar{x}_{e_3}$ = 1057.55
$\bar{x}_{e_4}$ = 54.96
$\bar{x}_{e_5}$ = 38.14
$\bar{x}_{e_6}$ = - 9.18
$\bar{x}_{e_7}$ = 18.59
e_i = 228.51

I(c): FISHER LSD TEST

$$LSD = \begin{cases} 358.50 & \alpha = 0.05 \\ 571.41 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_2}$	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_7}$	$\bar{x}_{e_1}$	$\bar{x}_{e_6}$
$\bar{x}_{e_3}$	620.38**	1002.59**	1019.41**	1038.96**	1055.18**	1066.73**
$\bar{x}_{e_2}$		382.21*	399.03*	418.58*	434.80*	446.35*
$\bar{x}_{e_4}$			16.82	36.37	52.59	64.14
$\bar{x}_{e_5}$				19.55	35.77	47.32
$\bar{x}_{e_7}$					16.22	27.77
$\bar{x}_{e_1}$						11.55

II(a): LINK-WALLACE ANOVA

Ranges: $R_1 = 6.740$, $R_2 = 1568.96$, $R_3 = 2421.8$, $R_4 = 1583.8$, $R_5 = 136.84$, $R_6 = 2594.6$, $R_7 = 3593.$, $R_{\bar{x}_{e_i}} = 1066.727$, $\sum_i R_i = 11905.740$

From tables (n=13, k=7): $K_{n,k;\alpha} = \begin{cases} 0.66 & \alpha = 0.05 \\ 0.79 & \alpha = 0.01 \end{cases}$

$$K = \frac{(13)R_{\bar{x}_{e_i}}}{\sum R_i} = 1.165^{**}$$

II(b): LINK-WALLACE MULTIPLE COMPARISONS

$$D = \frac{K_{n,k;\alpha} \sum R_i}{n} = \begin{cases} 604.445 & \alpha = 0.05 \\ 723.503 & \alpha = 0.01 \end{cases}$$

	$\bar{x}_{e_2}$	$\bar{x}_{e_4}$	$\bar{x}_{e_5}$	$\bar{x}_{e_7}$	$\bar{x}_{e_1}$	$\bar{x}_{e_6}$
$\bar{x}_{e_3}$	620.38*	1002.59*	1019.41*	1038.96*	1055.18*	1066.73*
$\bar{x}_{e_2}$		382.21	399.03	418.58	434.80	446.35
$\bar{x}_{e_4}$			16.82	36.37	52.59	64.14
$\bar{x}_{e_5}$				19.55	35.77	47.32
$\bar{x}_{e_7}$					16.22	27.77
$\bar{x}_{e_1}$						11.55

III(a): FRIEDMAN TEST

Compartment #	Sum of Ranks
1	29
2	63
3	79
4	56
5	45
6	44
7	48

$\hat{\chi}^2_r = 25.12^{**}$

III(b): WILCOXON & WLICOX TEST

From tables: $D_{ww;\alpha} = \begin{cases} 32.5 & \alpha = 0.05 \\ 38.0 & \alpha = 0.01 \end{cases}$

	2 (63)	4 (56)	7 (48)	5 (45)	6 (44)	1 (29)
3 (79)	16	23	31	34*	35*	50**
2 (63)		7	15	18	19	34*
4 (56)			8	11	12	27
7 (48)				3	4	19
5 (45)					1	16
6 (44)						15

* indicates significance at $\alpha = 0.05$
** indicates significance at $\alpha = 0.01$

CHAPTER 5

CONCLUSION

5.0 INTRODUCTORY REMARKS.

In the previous chapters we have discussed discrete and continuous models and their estimation, bilinear time series, difference equations with random slope and intercept, diffusions, etc. Both the processes generated in chapter 3 and estimated in chapter 4 are Markov, and random walks (e.g., the Markov chain of the discrete model) converge to diffusions (of the continuous model) under certain conditions. The question still remains how are the two models, discrete and continuous, related.

Considering only the discrete model: statisticians tend to look at time series as made up of seasonal effects, trends, and other 'irregular'

fluctuations. On the other hand, engineers see the same thing as having a transient and a steady state component. Probably, the two approaches are equivalent. This thesis has been from the latter point of view (e.g., with respect to asymptotic stationarity).

5.1 RELATIONSHIP BETWEEN DISCRETE AND CONTINUOUS MODELS.

This work has been an attempt to model a population of lymphocytes, the recirculating lymphocyte pool, portions of which are subjected to seven different environments, the compartments. For the moment assume that it is a homogeneous population, the justification for this being that RLP is overwhelmingly T-cells. There is a distinct growth rate in each environment. Thus the population can be modeled by a 7-state Markov chain. In discrete time, products of stochastic matrices and, in continuous time, random evolutions may be viewed as representing such a population dynamics. The relationship of discrete and continuous multiplicative processes is discussed in Appendix 1. Other points of view are discussed below.

5.1.1 CONVERGENCE OF THE DISCRETE TO THE CONTINUOUS MODEL.

Stochastic differential equations are formal expressions for stochastic integral equations, so that the interpretation of the former depends on that of the related latter equations. Stochastic integrals can be defined in different ways, two of which are touched on in Appendix 4, which may be referred to for more details, if needed. Up to now, in this thesis, only Ito type stochastic differential equations have been used.

One way of looking at the discrete model is as an Euler's approximation (neglect the additive noise for the time being)

$$x(n+1) = x(n) + \frac{\tau}{N}\tilde{A}\, x(n) + \sum_i B_i\, x(n)\, \Delta W^i(n) \qquad \ldots(5.1)$$

of the homologous continuous equation

$$(S) \quad dx(t) = \tilde{A}\, x(t)dt + \sum_i B_i\, x(t)\, dW^i(t) \qquad \ldots(5.2)$$
$$t \in [0,\tau]$$

where

N = # steps in $[0,\tau]$,

n = # of the present step making
distance from zero $n\tau/N$,
(S) denotes Stratonovich representation.

Convergence of (5.1) to (5.2) has been discussed in literature (Rumelin, 1982; Clark & Cameron, 1980; Clark, 1985). The rate of convergence depends on the structure of B , i.e., whether they are commutative or not. In the non-commutative case

$$E|x(t) - x(N)|^2 = O(1/N) , \quad ...(5.3)$$

while in the commutative case

$$E|x(t) - x(N)|^2 = O(1/N^2) . \quad ...(5.4)$$

(Pardoux & Talay, 1985; Thm 2.7 on p.36). In view of this, the discrete model (without the additive noise) converges to a continuous equation having Stratonovich form. This can be converted to the Ito form as noted above. Thus the relation between the parameters of the discrete model and those of the continuous model is such that: i) the coefficients, B_c, of the stochastic (integral) term are the same as for the difference equation, and ii) the coefficients, A^c (superscript

for Continuous), in the non-stochastic term are functions of the deterministic coefficients, A^D, (superscript for Discrete) and a correction term based on B_i.

During simulation, pseudo-random number generators are used instead of Wiener processes. The convergence of (5.1) using such replacement processes is discussed in Janssen (1984),and indeed there may be problems.

5.1.2 EMBEDDING.

For a hypothetical observer of the physiological process, (5.1) generates a Markov product of stochastic matrices induced by a Markov random evolution in (5.2). Looking at the respective mean processes:

$$E[x(n+1)] = (I + \tilde{A})\ E[x(n)] = A^D\ E[x(n)] \qquad \ldots(5.5)$$

and

$$E[\dot{x}(t)] = \tilde{A}\ E[x(t)] = A^c\ E[x(t)] \qquad \ldots(5.6)$$

The matrices A^D and A^C, for closed compartmental systems, are related by (Eisenfeld, 1979)

$$A^D = \exp(A^C) \qquad \ldots(5.7)$$

Similar results are presented in Appendix 1 for multiplicative processes. But we know from Kingman (1962) that a Markov chain that can be expressed by (5.7) is embeddable in a continuous process. Thus the mean discrete process can be embedded in the mean continuous process.

5.1.3 INVARIANCE OF MEASURE OF GROWTH RATE?

In the long run some measure of growth rate that is invariant under the two representations is desired (because the same physical process is being modeled differently). Cohen (1979) has shown that for a single population exposed to different environments and obeying (5.1) and (5.2) growth rates, λ_c, λ_D, defined as follows are the same under certain assumptions

$$\ln \lambda_c = \lim_{t \to \infty} \frac{1}{t} E[\ln M^c(t)] \qquad \ldots(5.8)$$

and

$$\ln \lambda_D = \lim_{n\to\infty} \frac{1}{n} E[\ln M^D(n)], \qquad \ldots(5.9)$$

where scalars $M^C(t)$ and $M^D(n)$ are thought of as population sizes respectively at time t in the continuous case and at instant n in the discrete case.

The present study can be seen as a multitype population in the Cohen sense (many populations exposed to many different environments simultaneously) and (5.8) and (5.9) must be modified by replacing scalars M^C and M^D by some scalar functions of A^C and A^D respectively. Such functions could be the norms $||A^C||$ and $||A^D||$ of the respective matrices. If these are used then in general,

$$\lambda^C \neq \lambda^D \qquad \ldots(5.10)$$

5.1.4 ARE THE PARAMETERS RELATED?

It is possible to relate the deterministic portion of the discrete and continuous models through relationships discussed in sections 5.1.1 and 5.1.2.

Here A^D is a first-order approximation of $\exp(A^c)$

$$A^D = \exp(A^c) = I + A^c + \ldots \qquad \ldots(5.11)$$

The sample paths generated by (5.1) and (5.2) cannot really be compared because even when they assume equal values at certain time instants, they obey different probability laws in between these instants

5.2 THE RESIDUAL ERRORS & THEIR STATISTICAL ANALYSIS

There are seven compartments with 13 residuals in each. Thus in effect these residuals have a multivariate distribution with 7 variables each with 13 sample points. In order to check the Gaussianity of the residuals instead of using the mutivariate distribution, a univariate test was done for convenience. All the 91 residuals were treated as being generated by a single population and their skewness and kurtosis tested for significance by procedures described in section 4.3.6. The results are presented in Table 21. All the residuals are non-Gaussian based on these tests except for those in second moments for the discrete case in which case the results of the Friedman test show

existence of at least two different distributions. This is a contradiction, so that in this case also it would be assumed that the errors are not Gaussian. This would be expected physically since the number of cells cannot be negative which implies that the distribution of residual errors will have a finite lower limit. Physiologically, recirculation in all the organs cannot be independent. Thus the usual assumptions of independence and normality do not hold. Robustness of the F-test is also in question because the data is based on only 5 rats for the most part. (See section A7.6 for comparison of parametric and non-parametric tests). Thus the Friedman test is the most appropriate test. Use of other (e.g. parametric) techniques is not only out of place, it leads to wrong conclusions. Three types of analyses were done here to demonstrate this. Even with relaxation of the independence assumption in the classical ANOVA and the use of Link-Wallace test leads to wrong conclusions, because it is difficult to state categorically how closely the distributions of residuals in each compartment (with size 13) approximates a Gaussian distribution.

Table 21

(a) RESULTS OF THE SKEWNESS TEST

WLS = Weighted Least Squares estimation
-2LL = -2 Ln Likelihood estimation

(VALUES OF THE COEFFICIENT OF SKEWNESS)

	WLS	-2LL
DISCRETE:		
1st Moments	-1.18**	-1.19**
2nd Moments	-0.69**	-0.72**
CONTINUOUS:		
1st Moments	1.50**	1.49**
2nd Moments	0.36	0.34

(b) RESULTS OF THE KURTOSIS TEST

(VALUES OF KURTOSIS)

	WLS	-2LL
DISCRETE:		
1st Moments	5.22**	5.38**
2nd Moments	3.51	3.62
CONTINUOUS:		
1st Moments	7.04**	7.00**
2nd Moments	4.09*	4.12*

* denotes significance at $\alpha = 0.05$
** denotes significance at $\alpha = 0.01$

5.3 BIOLOGICAL INTERPRETATION OF THE RESULTS OF THE ANALYSES IN CHAPTER 4.

5.3.1 THE DISCRETE MODEL.

Stability: Like every closed discrete (stochastic) system the mean number of lymphocytes in the different compartments are in marginal equilibrium (spectral radius of $\hat{A}$, $\rho(\hat{A}) = 1$). Since no births and deaths are assumed (conservation of matter) a slight disturbance can cause the estimated mean values to oscillate. For the estimated values of the noise variances (or standard deviations) the system moves away from equilibrium (see section 4.4.2).

To investigate this situation, the analyses mentioned above were done to see how well the model fits the given data and which compartment(s), if any, contributed most to the discrepancy. It is expected that for a model that fits the data closely, the residual errors should not be significantly different from zero and be more or less symmetrically distribed around it for very small errors. With increasing size of errors there will be increasing asymmetry in the distribution with a positive skew. It turned out that

in both weighted least squares and -2 ln likelihood estimations, the Friedman's two-way analysis of variance rejected the null hypothesis that the residuals in all the compartments are from the same populations. Multiple comparisons divided the compartments into the following subsets:

1st moments: {(1,3,4,5,6,7),(2,6)}
2nd moments: {(1,2,3,5),(1,2,5,6,7),(4,6,7)}.

As seen in Table 21, the distribution of the residual errors in the first moment has a negative skew. Most of the points in the subset (2,6) are negative, while those in the subset (1,3,4,5,6,7) are more symmetrically distributed and are more likely to contain the zero point. Compartment # 6 being in both subsets has a distribution of lymphocytes that is similar to one subset in some respects and to the other subset in other respects. Thus the more likely source of, or at least of greater part of the discrepancy seems to be compartment # 2 (spleen). For the size of residual errors refer to Tables 4 and 5.

Similarly the residuals in variances (actually the 2nd moments) are being pulled to the positive side by those in compartment # 3 (other tissues) and toward

the negative side by those in compartment # 4 (LN-a). Since LN-a drain the 'other tissues', any error in the model of compartment # 3 also shows up in compartment # 4.

Thus to get a model which approximates the real-world system more closely it would seem necessary to improve the equations for the spleen and the 'other tissues'.

5.3.2 THE CONTINUOUS MODEL.

Stability: Like every closed compartmental system the mean number of lymphocytes in the different compartments are in marginal equilibrium (spectral radius of $\hat{A}$, $\rho(\hat{A}) = 0$). Just as in the discrete case the system model being conservative will tend to oscillate on a slight perturbation caused by the parametric noise. Again for the estimated noise variances the system model will move away from equilibrium.

Analyses of residual errors from both weighted least squares and -2 ln likelihood estimations yielded the same results. The Friedman test again rejected the

null hypothesis that the residuals in all compartments are drawn from the same populations. Multiple comparisons gave the following compartment subsets:

1st moments: {(1,3,4,5,7),(1,4,5,6,7),(1,2,5,6,7)}
2nd moments: {(2,3,4,7),(2,4,5,6,7),(1,4,5,6,7)}.

Using reasoning similar to that for the discrete model, here also the residuals for the means are being pulled to the positive side by errors in compartment # 3 (Other tissues) and to the negative side by those in compartment # 2 (the spleen), but the errors in # 3 are far bigger resulting in the positive skew in Table 21. In the case of the residuals in variances (2nd moments) one might expect compartments # 1 and # 3 to be the main source of distortion.

Thus it is suggested that equations for the bone marrow (compartment # 1), spleen (# 2), and the other tissue (# 3) need to be improved.

5.4 VALIDITY OF THE MODELS.

It has just been seen that there is a lack of fit between the data and the models. Does this mean that

the models are not valid?

5.4.1 ARE THE MODELS VALID ?

Validity was mentioned earlier also (in section 4.3). It was defined as: a model is considered VALID if its performance in terms of its output is similar to that of the relevant real-world system when the same inputs are applied to both. Validity holds only over a certain range of parameters and only for the specific function/process the model was designed for. Borrowing ideas from psychometrics, different types of validity can be looked at. Two types will be defined: content validity and construct validity.

Content validity is the type of validity that can be established through a rational analysis of the structure of the model. It's determination is based on individual, subjective judgement. If a model is logically consistent based on the real system it has content validity. The author audaciously enough claims fairly high degree of content validity for the models developed in chapter 3. It was not just a matter of fitting any odd curve to the data, in which case content validity would be zero. The models were developed by

more or less thorough logical physico-physiological analysis, but the approximations introduced to model only a specific, selected function of the real system together with the assumptions to simplify the model structure attenuate the validity.

Construct validity is the degree to which the model mimics the theoretical function or process that it was designed to mimic. Hopes for construct validity for the models in this study are dashed by significance of $\hat{\chi}^2_R$ in all Friedman tests. This means that the following alternative conclusions may be drawn: i) the experiment being modeled was flawed, ii) the reasoning used in developing the models was at least partially wrong or incomplete and should be revised, or iii) the equations are totally invalid for the process.

If the equations were incorrect for the particular process to be modeled they would have to be replaced, but content validity here advises against that. For the experiment being flawed, there will be a mention of flaws in the available data, and for partially incomplete reasoning there will be a discussion about the contribution of violation of assumptions that were made and of the estimation algorithms used.

5.4.2 DATA.

The sparsity of data was discussed in more detail previously in section 4.1. Primarily means and variances are available with some values missing. No covariances are available (This is also because of the nature of the experiment: since rats are sacrificed at each time point; it is impossible to obtain longitudinal data over time).

5.4.3 VIOLATION OF ASSUMPTIONS.

Throughout this study many assumptions were made to simplify the model and the analyses. Many of these are violated in real life.

i) Homogeneity of organs --- it was assumed that many organs (e.g. LN) were structurally and physiologically the same so that they could be lumped together as a single compartment.

ii) Independence of events --- The body is a whole of mutually interacting systems, even small pertubations in different regions though seemingly independent may not be so. (This assumption was made when lumping compartments.)

iii) Gaussianity --- it was shown above (section

5.2.7) that the residual errors are not Gaussian. This implies that the stochastic processes that were involved in the difference (the experimental state and the estimated state) are non-Gaussian.

iv) Stationarity --- In order to test if the distribution of residuals changes over time, Friedman test was performed on the transposed matrix of residuals (interchanged rows and columns), taking time as the treatment variable and compartments as the independent variable. By so doing, n and k interchange values in formula (A7.2) and the rest of the procedure is as described before. Testing thus reveals (see Table 22) that only 2nd moments in continuous case show significance (at $\alpha = 0.01$). We must qualify all statements made about our models with the phrase "over the period of observation".

v) There are births and deaths. Births can be neglected for the labelled cells; death (about 2% daily) cannot be. To be very precise what we have modeled is distribution of radioactivity (the tracer) and not that of the cells themselves. Also, over long time some label is lost from the cells.

vi) Assumptions in compartmental analysis are violated. e.g. indistinguishable particles, well-mixed compartments, and same probability of

transition.

vii) Existence of different types of immunities was assumed. Also it was assumed that they remain constant. This may not be a very valid assumption (see section 1.2).

viii) To be very precise distribution of the radiolabel was modeled here, and not that of the cells. The latter was modeled only in so far as the label was not lost, and births and deaths did not occur.

Table 22

FRIEDMAN TEST FOR STATIONARITY

WLS = Weighted Least Squares estimation
-2LL = -2 Ln Likelihood estimation

I = Discrete Model, 1st Moments, WLS
II = Discrete Model, 1st Moments, -2LL
III = Discrete Model, 2nd Moments, WLS
IV = Discrete Model, 2nd Moments, -2LL
V = Continuous Model, 1st Moments, WLS
VI = Continuous Model, 1st Moments, -2LL
VII = Continuous Model, 2nd Moments, WLS
VIII = Continuous Model, 2nd Moments, -2LL

I

Time instant	Sum of Ranks
1	48
2	38
3	39
4	45
5	48
6	45
7	55
8	55
9	55
10	56
11	52
12	50
13	51

χ^2_4 = 4.01

II

Time instant	Sum of Ranks
1	48
2	38
3	38
4	45
5	48
6	45
7	55
8	55
9	55
10	56
11	52
12	50
13	52

χ^2_4 = 4.26

III

Time instant	Sum of Ranks
1	26
2	46
3	55
4	59
5	55
6	52
7	59
8	59
9	53
10	51
11	43
12	44
13	35

χ^2_4 = 11.27

IV

Time instant	Sum of Ranks
1	26
2	46
3	55
4	59
5	55
6	52
7	59
8	59
9	53
10	51
11	43
12	44
13	35

χ^2_4 = 11.27

V

Time instant	Sum of Ranks
1	47
2	36
3	42
4	41
5	47
6	42
7	56
8	55
9	56
10	58
11	55
12	48
13	54

χ^2_4 = 5.80

VI

Time instant	Sum of Ranks
1	47
2	36
3	42
4	42
5	47
6	41
7	56
8	55
9	56
10	58
11	54
12	49
13	54

χ^2_4 = 5.69

VII

Time instant	Sum of Ranks
1	19
2	23
3	50
4	57
5	61
6	64
7	69
8	65
9	60
10	65
11	54
12	38
13	12

χ^2_4 = 42.93**

VIII

Time instant	Sum of Ranks
1	19
2	24
3	50
4	57
5	61
6	64
7	69
8	65
9	60
10	65
11	54
12	37
13	12

χ^2_4 = 42.67**

SUMMARY OF THE TEST STATISTICS
(Values of χ^2_4)

	WLS	-2LL
DISCRETE:		
1st Moments	4.01	4.26
2nd Moments	11.27	11.27
CONTINUOUS:		
1st Moments	5.80	5.69
2nd Moments	42.93**	42.67**

* denotes significance at α = 0.05
** denotes significance at α = 0.01

5.4.4 ALGORITHMS USED.

Because nonlinear optimization computation was performed it is impossible to say if the minima reached on the error surfaces were global or local. Convergence properties of the weighted least squares and the -2 ln likelihood procedures used were not studied.

Parameter estimation procedures used here can be considerably improved on.

5.5 SUGGESTIONS.

i) In the experiment that has been modeled here obtaining temporally sequential data is not possible. It would be useful to have raw data available for each organism rather than mixed data or only the summary of descriptive statistics. Also more data is needed --- just 13 data points is not enough and though it becomes expensive and time consuming more rats are also needed (just 4 or 5 at each time point are hardly sufficient).

ii) In the estimation and simulation non-Gaussian distribution should be used (any non-negative distribution, e.g. lognormal or positive gamma distribution). Physically, this is reasonable because

the process cannot have negative values.

iii) It is known that in real-life situations within a certain range the coefficient of variation (ratio of standard deviation to the mean = σ/μ) remains more or less constant. In many cases, organisms have been reported to have a particular coefficient of variation over a certain range, sometimes of other parameters, (e.g. coefficient of variation relative to height or length of an organism over a certain age, in particular the growing period). This has sometimes been used to check the validity of data obtained from experiments (Snedecor & Cochran, 1968). It may be useful in modeling also, to check if the model mimics the data.

iv) Recurrence properties of the models developed here were not studied. It would be interesting to look at the mean residence times and mean recurrence times for the compartments. The residence times could be compared with "delay times" in the compartments.

v) The continuous model should also have an additive noise term to account for random variations between organisms.

vi) A death term should be included. 2% of

lymphocytes die daily and are disposed of by the liver. This would damp out the marginal instability.

vii) Estimation using more data should give better parameter estimates. But if there is still some discrepancy and it is because of compartments 2 and 3 in the discrete case and compartments 1, 2, and 3 in the continuous case (as in section 5.3) then the following modifications in equations would be advised.

BONE MARROW (Compartment # 1): Waugh et.al. (1984) have presented an analysis of the physical factors involved in reticulocyte egress from bone marrow and the active nature of leukocyte egress. Factors discussed there (e.g. viscosity of WBC cytoplasm, pore size in marrow, viscosity of surrounding fluid, WBC membrane properties) may be incorporated in the continuous model.

SPLEEN (Compartment # 2): Hammond (1975) modeled lymphocyte circulation in the spleen. An overall model of the spleen based on his data may be used to modify the equations here in both discrete and continuous cases.

OTHER TISSUES (Compartment # 3): This compartment includes lymphoid tissues as liver, gut,

and peyer's patches and miscellaneous non-lymphoid tissues (e.g. skin). This is a heterogeneous group in all respects (structurally and functionally). It may be better to divide this either into two sub-compartments consisting of lymphoid tissues and non-lymphoid tissues or into three sub-compartments comprising non-lymphoid tissue, liver, and gut and peyer's patches. In the latter division it would be possible to include the 3 separate functions of the liver related to recirculation, namely, a) genuine recirculation (from blood to liver to coeliac LN, thoracic duct and back to blood), b) intravascular pooling (similar to lungs resulting in a rapid initial response, and iii) accumulation of dying and dead cells.

Ford's data does not include the non-lymphoid sub-compartment. No variances are available and means have only been deduced from his data for this sub-compartment. Thus least amount of information is available about it and when lumped with other organs again creates a compartment with strangely mixed and incomplete information (relative to other compartments). It is logical that compartment 3 is a bad fit in both discrete and continuous cases.

5.6 CONCLUSIONS.

Models of different functions of the immune system are available. Some of them were discussed in section 1.3. One of the ways in which the different functions interact is through recirculating lymphocytes (section 2.1.6 and 2.1 in general). The present project was an attempt to model the distribution of recirculating lymphocytes. Best available published data (section 2.2) was used as the basis for the model, but it was not sufficient. For this and other reasons discussed in section 5.4 the model did not fit the data.

It would be worthwhile to obtain more data and try to estimate the parameters using the same models. If it still does not fit, then changing it structurally as suggested in section 5.5.(vii) would be indicated. It would also be useful to include another noise term: the process of observation causes distortion in the system and should be taken into account.

So far as analyses are concerned first the Friedman test (with very few assumptions) should be done and the gaussianity of the data for analysis checked (using skewness, kurtosis tests or Kolmogorov-Smirnov test) and then more restrictive test done.

The models presented here are just a first step. A great deal of work still needs to be done to obtain models which approximate the real-world system more closely.

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APPENDICES

APPENDIX 1

MULTIPLICATIVE PROCESSES.

(Birkhoff & Varga, 1958)

A real square matrix $P=[p_{ij}]$ is called NONNEGATIVE ($P \geqslant 0$) iff $p_{ij} \geqslant 0, \forall i,j$. Similarly, a real square matrix $Q=[q_{ij}]$ is called ESSENTIALLY NONNEGATIVE iff $q_{ij} \geqslant 0, i \neq j$. A discrete multiplicative process with a finite number n of "states" is a system of difference equations

$$x_i(r+1) = \sum_{j=1}^{n} p_{ij}\, x_j(r) \quad i = 1,\ldots,n \quad \ldots(A1.1)$$

where P $(=[p_{ij}]) \geqslant 0$. A continuous multiplicative process is any system of ordinary differential equations

$$\dot{x}_i(t) = \sum_{j=1}^{n} q_{ij}\, x_j(t) \quad i = 1,\ldots,n \quad \ldots(A1.2)$$

where Q $(=[q_{ij}])$ is essentially nonnegative. These processes are supplemented by the conditions

$$\sum_{i=1}^{n} p_{ij} = 1 \quad j = 1,\ldots,n \quad \ldots(A1.3)$$

or

$$\sum_{i=1}^{n} q_{ij} = 0 \quad j = 1,\ldots,n \quad \ldots(A1.4)$$

respectively. A nonnegative matrix satisfying (A1.3) is called a column STOCHASTIC matrix. Some important properties of such matrices follow (Birkhoff & Varga, 1958).

LEMMA A1.1. The matrix Q defines a continuous Markov process (A1.2) and (A1.4) iff P = exp(Qt) defines a discrete Markov process (A1.1) and (A1.3) for any t > 0.

PROPOSITION A1.1. Any essentially nonnegative and irreducible matrix Q has a unique strictly positive eigenvector φ_1, with real simple eigenvalue $\lambda_1=M$. Moreover, $\lambda_1 > \mathrm{Re}\{\lambda_i\}$ for any other eigenvalue λ_i of Q.

PROPOSITION A1.2. Any irreducible, nonnegative matrix P has a unique strictly positive eigenvector φ_1, with positive simple eigenvalue $L=\mu_1$. Moreover, $\mu_1 \geq |\mu_i|$ for any other eigenvalue μ_i of P, and any nonnegative eigenvector is a scalar multiple of φ_1.

COROLLARY A1.1. The spectral radius of any

nonnegative irreducible matrix P is L in proposition A1.2; the spectral prenorm (Max of real parts of λ_i) of any essentially nonnegative irreducible matrix Q is M in proposition 1.1. The spectral radius of exp(Qt) is exp(Mt), if $t \geqslant 0$.

APPENDIX 2

RESULTS ON LINEAR TIME-INVARIANT COMPARTMENTAL SYSTEMS.

Some results on the nonnegativity, boundedness, connectivity, stability and identifiability of linear time-invariant (LTI) compartmental systems follow. For more details see the references cited.

A2.1 NONNEGATIVITY AND BOUNDEDNESS

PROPOSITION A2.1. (Birkhoff & Varga, 1958) A is essentially nonnegative iff $\exp(tA) > 0$, $t>0$.

PROPOSITION A2.2. (Birkhoff & Varga, 1958) $\mathrm{Exp}(tA) > 0$ for $\forall\ t > 0$ iff A is irreducible and essentially nonnegative.

PROPOSITION A2.3. (Hearon, 1963) $x(t) \geqslant 0$, $\forall\ t \geqslant 0$ iff $a_{ij} \geqslant 0$ $(i \neq j)$ in matrix A, while for $x(t) < \infty$ it is necessary that $a_{ii} < 0$.

These three propositions ensure the nonnegativity and the third the boundedness of the solution of (1.5).

A2.2 CONNECTIVITY

PROPOSITION A2.4. (Lancaster, 1969) A compartmental system (matrix A) is strongly connected iff the system matrix A is irreducible.

A2.3 EIGENVALUES OF A (STABILITY OF THE SYSTEM).

Knowledge of the eigenvalues of the system matrix is important in deducing the stability of the system. Some related results follow.

PROPOSITION A2.5. (Anderson, 1983) The real part of any eigenvalue of A is nonpositive. Moreover, A has no purely imaginary eigenvalue.

PROPOSITION A2.6. (Anderson, 1983) If A is a constant square matrix with column sums (or row sums) all equal to λ, then λ is an eigenvalue of A. Moreover, if $A \geq 0$, then λ is its spectral radius.

PROPOSITION A2.7. (Jacquez, 1972) A zero

eigenvalue occurs if, i) the system is closed, or ii) the system has a trap in it.

A2.4 IDENTIFIABILITY.

If the following are known: i) the system output function, ii) the number of compartments, iii) the connectivity of the system (i.e., which a_{ij} are zero), iv) the initial conditions, and v) the inputs, then the model is IDENTIFIABLE if all its a_{ij} $(i \neq j)$ can be uniquely determined as positive values from ideal or error-free data.

PROPOSITION A2.8. (Anderson, 1983) In order that a set of input-output data identify all a_{ij} it is necessary that i) all compartments be input reachable, and ii) all compartments possessing at least one path leaving them (including excretions) must be output reachable.

Anderson (1983) also gives several other necessary and sufficient conditions for identifiability.

APPENDIX 3

RESULTS ON LINEAR TIME-VARIANT COMPARTMENTAL SYSTEMS

PROPOSITION A3.1. (Mazanov, 1976) The solution of (1.6) is stable if

$$a_{ii}(t) \geqslant \sum_{\substack{j=1 \\ j\neq i}}^{n} a_{ji}(t) \quad i=1,\ldots,n; \ \forall t \ . \qquad \ldots(A3.1)$$

The solution remains stable if time-delays are introduced into the intercompartmental transfers.

PROPOSITION A3.2. (Mulholland & Keener, 1974)

1. (Nonnegativity) If $x_i(t)$ is a solution of (1.6) with $x_i(0) > 0$ for each $i = 1,2,\ldots,n$ then $x_i(t) > 0$ for all $t > 0$.

2. Suppose with $A(t)$ T-periodic (that is, with period T) and $\underline{u}(t) = \underline{0}$, (1.6) has no nontrivial periodic solution. Then with $\underline{u}(t) > \underline{0}$ and T-periodic, (1.6) has a unique T-periodic solution.

3. Suppose in (1.6) $u_i(t) = 0, \forall i$ and

$$a_{ii}(t) - \sum_{\substack{j=1 \\ j \neq i}}^{n} a_{ji}(t) \geq \delta > 0 \qquad \ldots(A3.2)$$

$$i = 1,\ldots,n \text{ and } a_{ij} \geq 0, \forall i,j.$$

If $x_i(t)$ is a solution of this system with $x_i(0) > 0$, $i = 1,2,\ldots,n$, then

$$\sum_{i=1}^{n} x_i(t) \leq \exp(-\delta t) \sum_{i=1}^{n} x_i(0) \qquad \ldots(A3.3)$$

4. Consider (1.6) with T-periodic A(t) and $\underline{u}(t)$, and A(t) diagonally dominant in the sense of (A3.2). Then the system (1.6) has a unique T-periodic solution to which all other solutions converge asymptotically.

APPENDIX 4

STOCHASTIC DIFFERENTIAL EQUATIONS

A4.1 REPRESENTATION OF STOCHASTIC DIFFERENTIAL EQUATIONS (Arnold, 1974).

Stochastic differential equations are formal expressions for stochastic integral equations, so that the interpretation of the former depends on that of the related latter equations. Stochastic integrals can be defined in different ways, two of which will be touched on now. Consider the integral

$$\int_{t_o}^{t} G(s,W_s)dW_s \quad . \qquad \ldots(A4.1)$$

Let

$$t_o \leq t \leq \ldots \leq t_n = t,$$

be a partition of (t_o,t). Define

$$S_n = \sum_{i=1}^{n} G(\tau_i, W_{\tau_i})(W_{t_i} - W_{t_{i-1}}) ,$$

where $t_{i-1} \leq \tau \leq t_i$. Then in the quadratic-mean limit

$$\int_{t_0}^{t} G(s, W_s)\, dW_s = \underset{\delta_n \to 0}{\text{qm-lim}}\ S_n ,$$

where

$$\delta_n = \max_i\ (t_i - t_{i-1})$$

and the location of τ_i can be expressed as

$$\tau_i = (1-a)t_{i-1} + at_i ,$$
$$0 \leq a \leq 1,\ i=1,2,\ldots,n$$

If a = 0, the integral in (A4.1) is referred to as the Ito integral, while if a = 1/2 it is referred to as Stratonovich's integral. Rules of ordinary calculus can be formally used for Stratonovich equations, but a special calculus is required for Ito equations. The Ito and Stratonovich forms can be transformed into each other. If the Stratonovich equation is written as (S in parentheses indicating Stratonovich representation)

$$(S) \quad dx_t = f(t, x_t)\, dt + G(t, x_t)\, dW_t , \quad .(A4.2)$$

then the corresponding Ito equation is (parenthetical I for Ito form)

$$(I)\quad dx_t = (f(t,x_t)+(1/2)\sum_{j=1}^{m}\sum_{k=1}^{n}(G_{x_k}(t,x_t)_{\cdot j}\,G(t,x_t)_{kj})dt + G(t,x_t)dW_t \qquad ...(A4.3)$$

where the n-vector $(G_{x_k})_{\cdot j}$ is the j-th column of the $n\times m$ matrix $G_{x_k}=[\partial G_{ij}/\partial x_k]$.

A4.2 MOMENTS OF LINEAR STOCHASTIC DIFFERENTIAL EQUATIONS.

The moments of the solution of (3.31) must satisfy the following result.

PROPOSITION A4.1. For the solution of (3.31), under the assumption that $E|c|^2 < \infty$, $c = x_{t_o}$,

i) $E[x_t] = m_t$ is the unique solution of

$$\dot{m}_t = A(t)\,m_t + a(t), \quad m_{t_o} = Ec \qquad ...(A4.4)$$

ii) $E[x_t x_t'] = P(t)$ is the unique nonnegative symmetric solution of

$$\dot{P}(t) = A(t)P(t) + P(t)A'(t) + a(t)m_t' + m_t a'(t) + \sum_{i=1}^{m} (B_i(t)P(t)B_i'(t) + B_i(t)m_t b_i'(t) + b_i(t)m_t' B_i'(t) + b_i(t)b_i'(t)) \quad \ldots(A4.5)$$

with initial value $P(t_o) = Ecc'$.

Proof. See theorem 8.5.5 in Arnold (1974).

A4.3 SMALL PERTURBATIONS IN DYNAMICAL SYSTEMS.

The following proposition applies to the continuous-time model (see section 3.6).

PROPOSITION A4.2. Let the components of vector-function $f(x,y)$ have continuous, bounded first and second partial derivatives throughout the whole space. Assume that the process ξ_t with values in $\mathbb{R}^m$ has piecewise continuous trajectories with probability one and fulfills the strong mixing condition with coefficient $\gamma(s)$ such that

$$\int_0^\infty s[\gamma(s)]^{1/5}\, ds < \infty$$

and

$$\sup_{x,t} E|f(x,\xi_t)|^3 < M < \infty .$$

Then as $\varepsilon \longrightarrow 0$ the process (of deviations) $\zeta_t^\varepsilon = (x_t^\varepsilon - x_t(\bar\theta))/\sqrt{\varepsilon}$ converges weakly on the interval $[0,\tau]$ to a Gauss-Markov process ζ_t° which satisfies the system of linear stochastic differential equations

$$d\zeta_t^\circ = A(x_t(\bar\theta),\bar\theta)\zeta_t^\circ dt + dW_t^\circ , \quad \zeta_0^\circ = 0 , \quad \ldots(A4.6)$$

where W_t° is a Wiener process and $A(x,\theta)$ is a square matrix such that

$$A(x,\theta) = [\partial f_i(x,\theta)/\partial x^j] ,$$

where $f_i(x,\theta)$ and x^j are the i-th and j-th elements of $f(x,\theta)$ and x respectively.

Proof. The proposition is stated without proof as a theorem in Marchuk et.al. (1986) who quote Venttsel & Freidlin (1979).

A4.4 SDE's AS DIFFUSIONS AND THE QUALITATIVE THEORY OF

SDE.

Under proper assumptions (Thm 9.3.1 of Arnold, 1974) SDE's can be thought of as diffusions. Autonomous SDE's can always be taken as homogeneous diffusions. Recent interest in the long-term behavior of stochastic systems has motivated development of a qualitative theory of SDE corresponding to the one for ODE. The qualitative theory of ODE (deterministic) deals with concepts like invariant sets, critical and periodic points, limit sets, recursive ideas (like recurrent/transient points), stability theory, etc. The corresponding theory for SDE deals with similar ideas (Arnold & Kliemann, 1983). There have been attempts at relating the properties of SDE's and their associated control equations (obtained by replacing noises with controls) (Brockett, 1976; Arnold & Kliemann, 1981). For recurrence and transience properties of diffusions see Bhattacharya (1978). He also discusses positive recurrence. Ferrante & Koch (1985) have examples of the use of Lyapunov functions to investigate qualitative properties of diffusion models, particularly in the two-dimensional case.

APPENDIX 5

BILINEAR TIME SERIES

Interest in real data has recently been reflected in an increased interest in robust methods (Martin, 1981; Preston, 1981; Franke, et.al., 1984) and irregularly observed data and/or missing observations (Jones, 1980; Jones, 1981; Dunsmuir, 1981; Parzen, 1984). Modeling using nonlinear time series is also receiving more attention now as is evident from Priestley (1981), who devotes one chapter (chapter 11) to nonstationarity and nonlinearity in his book. Other references include: Priestley (1980) and Haggan, Heravi, & Priestley (1984) who discuss state-dependent models, and Tjostheim (1986) and Pourahmadi (1986) who discusses doubly stochastic models. Recently there has also been a conference on nonlinear time series (Franke, Hardle, & Martin, 1984).

Bilinear systems are nonlinear and yet in some

respects resemble linear systems. They have been studied in the control and systems literature and many good references are available (Mohler, 1973; Bruni, DiPillo, & Koch, 1974; Mohler & Kolodziej, 1980a; Mohler & Kolodziej,1980b). Their application to biology and economics has been the subject of conferences, Mohler & Ruberti (1972), Ruberti & Mohler (1975), and Mohler & Ruberti (1978). More recently a class of bilinear time series models has been studied primarily in econometrics. Such models have the form of

$$\underline{x}(n+1) = A\underline{x}(n) + \sum_{j=0}^{m-1} B_j \underline{x}(n)\varepsilon(n-j) + C\varepsilon(n+1) , \qquad \ldots(A5.1)$$

where

$\underline{x}(n)$ = state at instant n

A = constant AR coefficient

B_j = constant mixed coefficients

C = constant MA coefficients

$\varepsilon(n)$ = noise

Granger & Anderson (1978) have discussed special homogeneous models of this type. Subba Rao (1981) found conditions for stationarity and discussed the problem of maximum likelihood estimation for a model of specified

order. Bhaskara Rao, Subba Rao, & Walker (1983) presented results on a multivariate strictly stationary process. More recently Subba Rao & Gabr (1984) have surveyed the area in their monograph, the first half of which is devoted to bispectral analysis and the second half to bilinear time series analysis. The latter, discussion includes Volterra series expansion, Markov representation, existence of bilinear time series, conditions of stationarity and invertibility, determination of order of the series, and estimation of the series. A model closely related to (A5.1) occurs in the literature under the name of random coefficient autoregressive model. The structure is

$$\underline{x}(n) = \sum_{i=1}^{m} \{A + \beta(n)\}\underline{x}(n-i) + \underline{\epsilon}(n) , \quad \ldots(A5.2)$$

where

$\underline{x}(n)$, A, $\underline{\epsilon}(n)$ are as for (3.14). $\beta(n)$ are also noises.

In (A5.2) $\{\underline{x}(n)\}$ is in effect a two-process bilinear time series.

Annals of Economic and Social Measurement (Vol. 2(4), 1973) devoted a special issue to time-varying parameters and included Rosenberg (1973) and three

sections dealing with i) random coefficient models, ii) systematic (non-random) variation models, and iii) Kalman filter models. Andel (1976) worked with scalar random coefficient autoregressive models and derived conditions of second-order stationarity. Spjotvoll (1977) surveyed the area, discussed estimating means and covariance matrices of the coefficients, and also statistical inference in finite samples. Nicholls & Quinn (1980) and Quinn & Nicholls (1981) discussed estimation of such models using least-squares and maximum likelihood estimates and studied their asymptotic properties. Nicholls & Quinn (1982) also have a monograph which surveys stability, stationarity, least squares and maximum likelihood estimation and the related asymptotic properties. Johnson (1977; 1980) provides annotated bibliographies for stochastic parameter regression which mainly deal with econometrics.

The general random coefficient autoregressive model of order m can be written as (Nicholls & Quinn, 1982)

$$\underline{x}(n) = \sum_{i=1}^{m} \{A_i + \beta_i(n)\}\underline{x}(n-i) + C\underline{\varepsilon}(n) \quad \ldots(A5.3)$$

where the variables are defined as the corresponding

variables in (A5.2).

Some properties of this structure will now be presented. Assumptions made during the theoretical analyses are followed by expressions for the solution and the first two moments, conditions for stability and stationarity, and conditions for existence and convergence of the second moment. Then follow properties of estimators for bilinear time series.

A5.1 ASSUMPTIONS.

The following assumptions will be made in the rest of the chapter concerning the discrete-time model (reference to equation 3.9 or to equation A5.3).

i) A_i, $i = 1,\ldots,m$ are constant $p \times p$ matrices,

ii) $\{\beta_i(n), n = 0,1,2,\ldots\}$, $i = 1,\ldots,m$ are i.i.d. sequences of pxp matrices such that $E[\beta_i(n)] = 0$ and $E[\beta_i(n) \otimes \beta_i(n)] = C_\beta$. $\otimes$ represents Kronecker product of two matrices, being defined such that the ij-th block of the product $A \otimes B$ is

$$[A \otimes B]_{ij} = a_{ij} B$$

where A and B are matrices.

iii) $\{\underline{\varepsilon}(n),\ n = 0,1,2,\ldots\}$, is an i.i.d. sequence of p-variate random vector with $E[\underline{\varepsilon}(n)] = 0$ and $E[\underline{\varepsilon}(n)\,\underline{\varepsilon}'(n)] = G$.

iv) $\{\beta_i(n)\}$ and $\{\varepsilon_i(n)\}$ are independent of one another.

The model as given in (3.9) is used here, and is quite similar to (A5.3) with the same assumptions. Since it is of first order ($m = 1$) the subscripts will be ommitted in (A5.3). Repeating,

$$\underline{x}(n+1) = A\underline{x}(n) + \beta(n)\underline{x}(n) + C\underline{\varepsilon}(n+1) \quad \ldots(3.9)$$

will be used in the rest of this appendix. In (3.9) A is a stochastic matrix (in the sense of Appendix 1) and $\beta(n)$ a closed compartmental matrix.

A5.2 RECURSIVE EXPRESSIONS FOR THE SOLUTION AND THE FIRST TWO MOMENTS.

A5.2.1 THE SOLUTION.

Equation (3.9) in itself is a one-step recursion. For

k-steps the expression is

$$\underline{x}(n+k) = \prod_{j=0}^{k-1}(A + \beta(n+k-j-1))\underline{x}(n) +$$

$$\sum_{i=0}^{k-2}\prod_{j=0}^{i}(A + \beta(n+k-j-1))C\underline{\varepsilon}(n+k-i-1) +$$

$$C\underline{\varepsilon}(n+k) \qquad \ldots(A5.4)$$

where $(A(j)) = A(0).A(1)...A(k)$

and for n-steps (i.e. the solution):

$$\underline{x}(n) = \prod_{j=0}^{n-1}(A + \beta(n-j-1))\underline{x}(0) +$$

$$\sum_{i=0}^{n-2}\prod_{j=0}^{i}(A + \beta(n-j-1))C\underline{\varepsilon}(n-i-1) +$$

$$C\underline{\varepsilon}(n), \qquad \ldots(A5.5)$$

$\underline{x}(0)$ being the initial condition.

A5.2.2 FIRST MOMENTS.

From (3.9), taking expectations:

$$E[\underline{x}(n+k)] = A\ E[\underline{x}(n+k-1)] \qquad \ldots(A5.6)$$

$$= A^k\ E[\underline{x}(n)] \qquad \ldots(A5.7)$$

A5.2.3 SECOND MOMENTS.

A5.2.3.1 VARIANCE.

From (3.9)

$$\underline{x}(n+1)\underline{x}'(n+1) = A\underline{x}(n)\underline{x}'(n)A' + \beta(n)\underline{x}(n)\underline{x}'(n)\beta'(n) + C\underline{\varepsilon}(n+1)\underline{\varepsilon}'(n+1)C' + \ldots\ldots$$

$$E[\underline{x}(n+1)\underline{x}'(n+1)] = AE[\underline{x}(n)\underline{x}'(n)]A' + E[\beta(n)\underline{x}(n)\underline{x}'(n)\beta'(n)] + CE[\underline{\varepsilon}(n+1)\underline{\varepsilon}'(n+1)]C'$$

$$\therefore \text{vec } E[\underline{x}(n+1)\underline{x}'(n+1)] = (A \otimes A + E[\beta(n) \otimes \beta(n)]).\ \text{vec } E[\underline{x}(n)\underline{x}'(n)] + (C \otimes C).\text{vec } E[\underline{\varepsilon}(n+1)\underline{\varepsilon}'(n+1)]$$

$$\therefore \text{vec } E[\underline{x}(n+1)\underline{x}'(n+1)] = (A \otimes A + C_\beta).\text{vec } E[\underline{x}(n)\underline{x}'(n)] + (C \otimes C).\text{vec } G, \qquad \ldots(A5.8)$$

where the vec operator transforms a matrix into a column vector by stacking its column. If

$$A = [c_1 \; c_2 \; \ldots \; c_p] \; ,$$

c_i being the i-th column of A, then

$$\text{vec } A = [c_1 \, , \; c_2 \, , \; \ldots , \; c_p]' \; .$$

The transposition, ', applies only to c_i and not to their elements.

The variance-covariance matrix, $E[\underline{x}(n)\underline{x}'(n)]$, being symmetric only the elements in its lower (or upper) triangle need to be considered to examine properties like stability. Thus instead of the vec operator the vech operator can be used. This operator stacks the columns of the lower triangular portion (including the diagonal) of a symmetric matrix. The vec and vech operators are related :

$$\text{vech } A = H \text{ vec } A \qquad \text{and} \qquad \text{vec } A = K \text{ vech } A$$

where if A is mxm, then vec A is $m^2 \times 1$, vech A is

$m(m+1)/2 \times 1$, H is $m(m+1)/2 \times m^2$, and K is $m^2 \times m(m+1)/2$. Expressed in terms of vech (A5.8) becomes

$$\text{vech } E[\underline{x}(n+1)\underline{x}'(n+1)] = H(A \otimes A + C_\beta)K.\text{vech } E[\underline{x}(n)\underline{x}'(n)] + H(C \otimes C)K.\text{vech } G, \quad \ldots(A5.9)$$

A5.2.3.2 MIXED SECOND MOMENTS.

$$\underline{x}(n+k)\underline{x}'(n) = \prod_{j=0}^{k-1}(A + \beta(n+k-j-1))x(n)x'(n) + \sum_{i=0}^{k-2}\prod_{j=0}^{i}(A + \beta(n+k-j-1)C\underline{\varepsilon}(n+k-i-1)\underline{x}'(n) + C\underline{\varepsilon}(n+k)\underline{x}'(n)$$

$$E[\underline{x}(n+k)\underline{x}'(n)] = E[\prod_{j=0}^{k-1}(A + \beta(n+k-j-1))\underline{x}(n)\underline{x}'(n)] = A^k E[\underline{x}(n)\underline{x}'(n)] \quad \ldots(A5.10)$$

It is of interest to investigate the conditions under which the solution of (3.9) (or (A5.3)) tends to an equilibrium independently of the initial conditions as $t \to \infty$ (stability), changes in the distribution

properties of parameters with time (stationarity), existence of moments, and recurrence properties.

A5.3 STABILITY.

Stability can be thought of as the property of reaching an equilibrium independently of the initial conditions. It is known that discrete systems are stable if the system matrix has eigenvalues inside the unit circle. This is more precisely stated below.

DEFINITION. $\{\underline{x}(n),\ n \in \mathbb{Z}^+\}$ generated by (3.9) is said to be stable if

$$\lim_{n\to\infty} E[\underline{x}(n)|\underline{x}(0)] = c_1 < \infty$$

and $$\lim_{n\to\infty} E[\underline{x}(n)\underline{x}'(n-s)|\underline{x}(0)] = c_2 < \infty$$

for fixed $s = 0,1,2,\ldots$ where c_1, c_2 are constants not depending on the initial value $\underline{x}(0)$.

PROPOSITION A5.1. The solution $\{\underline{x}(n),\ n \in \mathbb{Z}\}$ of (3.9) is stable iff $\rho(A \otimes A + C_\beta) < 1$.

Proof. Given in Conlisk (1974) for a similar model. Here $\rho(A)$ = spectral radius of A. Nicholls & Quinn (1982) have a similar condition. Spectral radius,

being maximum modulus of eigenvalues of a matrix has implications for stability of the matrix. In discrete case the system is stable when the spectral radius is less than unity. This means that all other eigenvalues are less than one. In proposition A5.1 the spectral radius of $(A \otimes A + C_\beta)$ is being considered. This matrix is composed of two second order terms, one of which depends on the deterministic portion of the transfer parameters and the other on the random portion. Thus stability is a function of the system matrix and the second moment of the multiplicative noise.

A5.4 SECOND-ORDER STATIONARITY.

Stationarity refers to the time-invariance of the distribution of a random variable. It is sometimes easier to consider time-invariance of the first two moments only. This is called second-order stationarity. For the particular model, (3.9), considered here, the covariance is a function of the variance and as such second-order stationarity can be stated precisely in terms of mean and variance only.

DEFINITION. The process $\{\underline{x}(n), n \in \mathbb{Z}\}$ generated by (3.9) is second-order stationary iff

i) the mean $\underline{\mu}_n = E[\underline{x}(n)]$, $n \geq 0$ is a constant,

ii) $Var[\underline{x}(n)]$, $n \geq 0$ is a constant, and

iii) the covariance $E[(\underline{x}(n)-\underline{\mu}_n)(\underline{x}(m)-\underline{\mu}_m)']$, $0 \leq m < n$ depends only on the interval $(n-m)$.

LEMMA A5.1. If $Var[\underline{x}(n)]$ is a constant, then the covariance $E[(\underline{x}(n)-\underline{\mu}_n)(\underline{x}(m)-\underline{\mu}_m)']$ is only a function of the time interval $(n-m)$, $0 \leq m < n$.

Proof. Obvious from (A5.10).

COROLLARY A5.1. If the mean, $E[\underline{x}(n)]$, and variance, $E[\underline{x}(n)\underline{x}'(n)]$, are constants, then $\{\underline{x}(n)\}$ from (3.9) is second-order stationary.

Proof. Obvious from Lemma A5.1 and the definition of second-order stationarity.

PROPOSITION A5.2. (Existence) Let assumptions i)-iv) in section A5.1 hold. For a asymptotically second-order stationary solution $\{\underline{x}(n), n \in \mathbb{Z}\}$ of (3.9) to exist it is necessary and sufficient that $\rho(A \otimes A + C_\beta) < 1$. This solution is given by

$$\underline{x}(n) = \sum_{i=0}^{\infty} \left(\prod_{j=0}^{i} (A + \beta(n-j-1)) \right) C\,\underline{\varepsilon}(n-i-1) + C\,\underline{\varepsilon}(n) \qquad \ldots(A5.11)$$

Proof. Can be derived as an extension of results

in Miller (1968). Also see Nicholls & Quinn (1982).

PROPOSITION A5.3. (Uniqueness) Let assumptions i)-iv) in section A5.1 hold. If a second-order asymptotically stationary solution $\{\underline{x}(n), n \in \mathbb{Z}\}$ to (3.9) exists and $\rho(A \otimes A + C_\beta) < 1$, then this solution is unique.

Nicholls & Quinn (1981; 1982) give many obvious results on the stationary values of first and second moments for singly-infinite stationarity (i.e. for $n \in \mathbb{Z}^+$) and many results similar on doubly-infinite stationarity ($n \in \mathbb{Z}$). Pham & Tran (1981) carry the same result for a bilinear process (their Theorem 2.2). From (A5.11) it appears that like a linear time series, (3.9) can be expressed as a 'moving average' process, in this case a 'two-process moving average process'.

A5.5 RELATION BETWEEN STABILITY AND SECOND-ORDER STATIONARITY.

The question is when the solution of time series in (3.9) tends to an equilibrium, do the distribution properties of the series change. The conditions for stability and second-order stationarity are the same so

they must be related. Intuitively, their definitions focus on the same concept from different points of view, stability emphasizing independence from initial conditions and second-order asymptotic stationarity constancy of the final values. The following proposition addresses this issue.

PROPOSITION A5.4. Let assumptions i)-iv) (section A5.1) hold. If $\{\underline{x}(n),\ n \in \mathbb{Z}\}$ is a stable solution of (3.9) then there exists a second-order stationary solution $\{\underline{x}^*(n)\}$ to (3.9) such that for fixed s

$$\lim_{n\to\infty} E[(\underline{x}(n) - \underline{x}^*(n))|\underline{x}(0)] = 0,$$

$$\text{and } \lim_{n\to\infty} E[(\underline{x}(n)-\underline{x}^*(n))(\underline{x}(n-s)-\underline{x}^*(n-s))'|\underline{x}(0)] = 0.$$

Proof. See Nicholls & Quinn (1982)

A5.6 STRICT STATIONARITY.

DEFINITION. The stochastic process $\{\underline{x}(n),\ n \in \mathbb{Z}\}$ generated by (3.9) is said to be strictly stationary if its finite-dimensional distributions are invariant under time displacements, that is, for t_i, t_i+t $[t_0,T]$,

$$F_{t_1+t,\ldots,t_n+t}(\underline{x}(1),\ldots,\underline{x}(n)) = F_{t_1,\ldots,t_n}(\underline{x}(1),\ldots,\underline{x}(n)).$$

F stands for distribution function.

Pham & Tran (1981) and Quinn (1982) present conditions for strict stationarity of $\{\underline{x}(n)\}$ generated by a bilinear model. The former give conditions similar to the ones mentioned above for a second-order process, while the latter obtains a strictly stationary ergodic solution to a first-order bilinear process if $E\{\ln|A+\beta(n)|\} < 0$ and only if $E\{\ln|A+\beta(n)|\} \leq 0$.

It must be cautioned that all the results presented here refer to asymptotic stationarity.

PROPOSITION A5.5. Suppose assumptions i)-iv) (section A5.1) hold. If a unique second-order stationary solution $\{\underline{x}(n), n \in \not\!\!Z\}$ exists to (3.9), then it is also strictly stationary and ergodic.

Proof. See Nicholls & Quinn(1982).

A5.7 EXISTENCE AND CONVERGENCE OF MOMENTS.

Granger & Anderson (1978) mention in passing that

all moments may not exist in the models that they have studied. In his paper, Tong (1981) states as corollary 1 that "for a non-trivial Markov bilinear process not all moments exist". For sufficiently large n, the n-th and higher order moments do not exist. If the (2i+2)th moment exists then the (2i+1)th moment also exists (i = 1,2,...). (Tong, 1981)

PROPOSITION A5.6. If, for the process $\{\underline{x}(n), n \in \mathbb{Z}^+ \text{ or } \mathbb{Z}\}$ in (3.9)

i) assumptions i)-iv) hold,

ii) $\rho(A \otimes A + C_\beta) < 1$,

then $E_\pi[\underline{x}(n)\underline{x}'(n)]$ exists

and $| E[\underline{x}(n)\underline{x}'(n)|\underline{x}(0)] - E_\pi[\underline{x}(n)\underline{x}'(n)] | = O(\eta^n)$, as $n \to \infty$ for some $\eta < 1$. π refers to a stationary measure.

Proof. See Thm 4 of Feigin & Tweedie (1985).

A5.8 ASYMPTOTIC PROPERTIES OF ESTIMATORS FOR THE DISCRETE MODEL.

A5.8.1 LEAST-SQUARES ESTIMATORS.

Pham & Tran (1981) have discussed this in relation to first-order bilinear time series

$$x(n) = ax(n-1) + b\varepsilon(n-1)x(n-1) + \varepsilon(n). \quad \ldots(A5.12)$$

They discuss invertibility of (A5.12), defining it as: the process $\{x(n)\}$ is invertible if $\varepsilon(n|x(0)) - \varepsilon(n)$ converges to zero in some sense as $n \to \infty$, regardless of $x(0)$. They find $\{x(n)\}$ from (A5.12) invertible or not according as $|b|$ is strictly less or strictly greater than $\exp\{-E[\ln|x(n)|]\}$.

Invertibility is closely related to parameter estimation. In the standard least squares method the sum of squares of 'errors', i.e. $\sum e^2_{\tilde{\theta}}(n|x(0))$ is minimized on some given set Θ to obtain parameter estimates $\theta = (a,b) \in \Theta$. $\tilde{\theta}$ indicates dependence on a guessed parameter set $(\tilde{a},\tilde{b})$.

Under the invertibility condition Pham & Tran minimize

$$V_n(\tilde{\theta}) = \frac{1}{n}\sum_{i=1}^{n} e^2(i|x(0)) \; . \qquad \ldots(A5.13)$$

They find (their Thm 4.1) that the least squares estimator $\tilde{\theta}(n)$ thus defined is strongly consistent.

For the random coefficient autoregressive models Nicholls & Quinn (1980; 1982) present results on strong consistency and distribution of estimates for scalar version of (A5.3). Under strict stationarity they show that $\hat{k} = [\hat{A}, \hat{\sigma}^2_\beta, \hat{\sigma}^2_\varepsilon]$ converges almost surely to $k = [A, \sigma^2_\beta, \sigma^2_\varepsilon]$ if $E[x^4(n)] < \infty$ and if $E[x^8(n)] < \infty$ the distribution of $N^{1/2}(\hat{k} - k)$ converges to a normal distribution with mean zero and a complicated covariance matrix (N being the total number of sample points). Nicholls & Quinn (1982) give a similar result in the multivariate case under similar conditions.

A5.8.2 MAXIMUM LIKELIHOOD ESTIMATORS.

Quinn & Nicholls (1981) and Nicholls & Quinn (1982) also discuss maximum likelihood estimation for scalar random coefficient autoregression. They assume strict stationarity and

$$E[\beta^4(n)] < \infty \text{ and } E[\varepsilon^4(n)] < \infty, \quad i = 1,\ldots,n.$$

and also appropriate bounds on covariances. In this way it can be shown that $\hat{\theta} = (\hat{A}, \hat{\sigma}^2_\beta/\hat{\sigma}^2_\varepsilon)$ converges almost surely to $\theta = (A, \sigma^2_\beta/\sigma^2_\varepsilon)$. Under the same conditions

$N^{1/2}(\hat{\theta} - \theta)$ converges to a normal distribution with zero mean.

Nicholls & Quinn (1982) while stating the algorithm to estimate parameters in the multivariate case, conjecture that here also the estimates "will be strongly consistent and satisfy a central limit theorem".

APPENDIX 6

MINIMIZING CRITERIA

The expression for weighted least squares cost function is:

$$Q_{\chi^2}(\theta) = \frac{1}{N}\sum_{i=1}^{k}\sum_{j=1}^{n}\frac{(x_{io}(j) - \hat{x}_i(j))^2}{\sigma_{io}^2(j)}$$

$$= \frac{1}{N}\sum(\underline{x}_o(j)-\underline{\hat{x}}(j))' \prod_o^{-1}(j)(\underline{x}_o(j)-\underline{\hat{x}}(j)) \quad , \qquad \ldots(A6.1)$$

where

$Q_{\chi^2}(\theta)$ = Chi-squared function to be minimized as a function of the parameter set being estimated,

$\underline{x}(j)$ = State at instant j (% activity),

$\sigma^2(j)$ = Variance at instant j,

$\prod(j)$ = Covariance matrix from observations at instant j (variances on the diagonal and zero off-diagonal),

θ = Parameter set; A for the first-moment equations, and (C_β, G) for the second-moment equations,

k = number of compartments (= 7),

n = number of observations per compartment (= 13),

N = total number of observations (= nk = 91),

cap $\hat{}$ denotes Estimate,

subscript i denotes Compartment i,

subscript $_o$ denotes Observation.

The analog of the Pearson χ^2-goodness-of-fit statistic is $N^*(Q_{\chi^2}(\theta))$. Thus to check the significance, it is more appropriate to use this statistic rather than $Q_{\chi^2}(\theta)$.

In the case of maximum likelihood estimation the residual errors are assumed independent so that if $f_\cdot(.|.)$ is the density of these errors then the likelihood function $L_N(\theta)$ is

$$L_N(\theta) = f_j(\underline{x}(1),\ldots,\underline{x}(j)|\underline{x}(0))$$

$$= \prod_{i=1}^{n} f_1(\underline{x}(i)|\underline{x}(0)) = \prod_{i=1}^{n}\prod_{m=1}^{k} f_1(x_m(i)|x_m(0)) \qquad \ldots(A6.2)$$

Logically a nonnegative asymmetric distribution (e.g., lognormal or gamma) should be used for $f.(.|.)$ in (A6.2). For the case of lognormal density function the maximum likelihood estimation equations are given in Cohen (1951). Alternately, assuming lognormal distribution the data can be transformed to a Gaussian distribution and the usual procedure as given below used. The mean ($\mu_i(j)$) and variance ($\sigma_i^2(j)$) for the transformed Gaussian distribution would be

$$\mu_i(j) = (1/2)\ \ln\left[\frac{x_{io}^4(j)}{\sigma_{io}^2(j)+x_{io}^2(j)}\right] \quad \text{and} \quad \sigma_i^2(j) = \ln\left[\frac{\sigma_{io}^2(j)+x_{io}^2(j)}{x_{io}^2(j)}\right]$$

For convenience, even though known to be invalid here, an underlying Gaussian distribution is assumed (as is usually done). Then (A6.2) is

$$L_N(\theta) = \prod_{i=1}^{n}\prod_{m=1}^{k}(2\pi\sigma_{mo}^2(i))^{-1/2}\ \exp\left(-\frac{1}{2}\cdot\frac{(x_{mo}(i)-\hat{x}_m(i))^2}{\sigma_{mo}^2(i)}\right)$$

Taking the logarithms,

$$\ln L_N(\theta) = -\frac{nk}{2}\ln(2\pi) - \frac{1}{2}\sum_{i=1}^{n}\sum_{m=1}^{k}\ln\ \sigma_{mo}^2(i) - \frac{1}{2}\sum_{i=1}^{n}\sum_{m=1}^{k}\frac{(x_{mo}(i)-\hat{x}_m(i))^2}{\sigma_{mo}^2(i)}$$

Let

$$l_N(\theta) = -\frac{2}{nk} \ln L_N(\theta) - \ln(2\pi)$$

$$= \sum_{i=1}^{n} \sum_{m=1}^{k} \ln \sigma_{mo}^2(i) + \frac{1}{nk} \sum_{i=1}^{n} \sum_{m=1}^{k} \frac{(x_{mo}(i) - \hat{x}_m(i))^2}{\sigma_{mo}^2(i)}, \quad \ldots(A6.3)$$

$$Q_{-2LL}(\theta) = l_N(\theta)$$

The $l_N(\theta)$ in (A6.3) is minimized using Powell's technique as mentioned earlier.

APPENDIX 7

RELEVANT STATISTICAL TESTS

Descriptions of the various tests are given in Appendix 7. Three types of statistical analyses were done: the classical one-way analysis of variance, Link and Wallace analysis of variance and Friedman's two-way analysis of variance. The three are compared in the appendix. Multiple comparisons that were done are also discussed. Terms used in the statistical literature are given in parenthesis in the description of the tests.

A7.1 THE CLASSICAL ONE-WAY ANALYSIS OF VARIANCE (ANOVA).

Assumptions:

In this procedure it is assumed that: i) observations within and between all compartments (random samples) are independent, and ii) the observations are from normally distributed populations with equal population variances.

How these assumptions affect the validity of the procedure is discussed below.

Hypotheses:

Because of the assumption ii), (4.2) can be written in terms of population means μ_i.

$$H_0 : \mu_1 = \mu_2 = \ldots = \mu_7 \quad \text{(null)}$$

$$H_1 : \text{All } \mu_i \text{ are not equal (alternative)}$$

...(A7.1)

Procedure: See any standard textbook of statistics for details. The calculations may be summarized in the form of a table as follows:

Source	SS	df	MS	F
Total	SS_3	df_3		
Between groups	SS_1	df_1	$MS_1 = SS_1/df_1$	$F = MS_1/MS_2$
Within groups	SS_2	df_2	$MS_2 = SS_2/df_2$	

where

Source refers to the source of the variance.

SS = Sum of Squares of deviations from the relevant mean,

df = Degrees of Freedom,

MS = Mean Sum of squares (= SS/df),

a measure of relevant sample variance,

F = the test statistic, having F-distribution when the samples are Gaussian,

$SS_3 = SS_1 + SS_2$,

$df_3 = df_1 + df_2$.

In this table groups refer to the compartments of this study. The F-test is used to decide if the null hypothesis is to be rejected. Since the F-test compares the sample variance between the compartments (groups) with the sample variance within the compartments (groups), the rejection occurs only when the former is significantly different from the latter in which case it can no longer be claimed that variance between groups was because of chance.

A7.2 LINK AND WALLACE ANALYSIS OF VARIANCE.

For our model it seems a bit far-fetched to assume the independence of all observations. The observations in different compartments and at different times are correlated. Thus a two-way analysis of

variance would be better. It could also detect any interaction between compartments and time, which intuitively seems evident to occur because of homing properties of lymphocytes. Here there is only one observation per cell (i.e., in a compartment at a time instant). In such a case a rapid test of analysis of variance according to Link and Wallace may be used (Sachs, 1984).

Assumptions:

It is assumed that individual samples in the compartments (groups) have an approximately normal distribution, identical variances, and the same sizes.

Hypotheses:

Same as for the classical ANOVA.

$$H_0 : \mu_1 = \mu_2 = \ldots = \mu_7 \quad \text{(null)}$$

$$H_1 : \text{Not all } \mu_i \text{ are equal (alternative)}$$

...(A7.1)

Procedure: The k ranges R_i of individual compartments (groups) and the range of means $R_{(\bar{x}_i)}$ are used. H_0 is rejected in favor of H_1, whenever

$$K = \frac{n\, R_{(\bar{x}_i)}}{\Sigma R_i} > K_{n,k;\alpha}$$

Special tables are available for the critical values of the test statistic K.

Here ranges serve as measures of variance of the samples, and the test is intuitively based on a principle similar to the one for classical ANOVA. Here $R_{(\bar{x}_i)}$ serves as a measure for between compartment sample variance and $\sum_i R_i/n$ as that for within compartment sample variance. If the former is too big compared to the latter the difference could be statistically significant (i.e., may not be because of chance). Not much is known of the sampling distributions of ranges. For the purposes of this test approximation of the distribution of range when considered a linear function of sample variance and other details of derivation are given in Kurtz, et.al. (1965).

A7.3 THE FRIEDMAN TEST (DOUBLE PARTITIONING WITH A SINGLE OBSERVATION PER CELL).

The Friedman test (or Friedman two way analysis of variance) is a non-parameteric analog of the classical (or parameteric) two-way analysis of variance and is used to compare several correlated samples

(Sachs, 1984) with respect to their central tendency.

Assumptions:

It is assumed that the samples have continuous distribution functions, F_i .

Hypotheses:

$$H_o : F_1 = F_2 = \ldots = F_T \quad \text{(null)}$$

$$H_1 : \text{All } F_i \text{ are not equal (alternative)}$$

...(4.2)

Procedure: The comparison is done between k compartments (treatments) with n time instants of measurement (individuals/samples) each. n rank orders are obtained by assigning a rank from 1 to k to every compartment (treatment). Thus the ranks, R_{ij} , of the data are represented in the following tabular form:

		Compartments (Treatments) 1	2	...	k	Row sums
	1	R_{11}	R_{12}	...	R_{1k}	k(k+1)/2
	2	R_{21}	R_{22}	...	R_{2k}	k(k+1)/2
Time	.	.....				.
Instants	.	.....				.
(Individuals)	.	.....				.
	n	R_{n1}	R_{n2}	...	R_{nk}	k(k+1)/2
Col.Sums		R_1	R_2	...	R_k	nk(k+1)/2

Thus the mean of the column sums is $n(k+1)/2$. If the sum of squares of column sums around this mean (denoted S) is found it can serve as a measure of between compartment (treatment) sample variance. As a measure of within compartment sample variance, the total sum of squares of deviations around the average rank (denoted s_t) is used. It turns out to be

$$s_t = nk(k-1)/12 .$$

The test statistic $\hat{\chi}^2_R$ is computed by, a linear function of S, $\hat{\chi}^2_R = (n-1)S/s_t$. After some algebra it becomes

$$\hat{\chi}^2_R = \frac{12}{nk(k+1)} \sum_i R_i - 3n(k+1) \qquad \ldots(A7.2)$$

The exact distribution of such a statistic for ranks is not known. For large k and large samples ($n > 7$), by comparing moments Gibbons (1985) approximates it by the χ^2-distribution with k-1 degrees of freedom. (For details see Gibbons, 1985.) Tables are available for the critical values of $\hat{\chi}^2_R$ for small n.

Corresponding to the table for the calculation of F-statistic in classical ANOVA (as in section A7.1), here usually only column number and column sums are

tabulated and the $\hat{\chi}^2_R$-statistic calculated from these. Other variables (n, k) in (A7.2) are known.

A7.4 MULTIPLE COMPARISONS.

When the null hypothesis is rejected we do not know distributions in which compartments (samples) differ and in which they do not. Multiple comparisons, however, are used to investigate this. Comparisons which were used with each of the above tests are described below.

A7.4.1 FISHER LSD (LEAST SIGNIFICANT DIFFERENCE) TEST.

This was used when H_o was rejected in the one-way ANOVA. It is a modified t-statistic, the critical value being

$$LSD_{\alpha} = t_{N-K;\alpha}\sqrt{\frac{2}{n}s^2_{error}} = \sqrt{\frac{2}{n}s^2_{error}\,F_{1,N-K;\alpha}} \quad \ldots(A7.3)$$

This is used to check the significance of all (k-1)k/2 pairwise comparisons.

The procedure used here is to arrange the

compartment means by order of magnitude

$$m_1 > m_2 > \ldots > m_7 ,$$

and then form the table

	m_2	m_3	...	m_7
m_1	d_{12}	d_{13}	...	d_{17}
m_2		d_{23}	...	d_{27}
.			...	.
.			..	.
m_6				d_{67}

Here the differences,

$$d_{ij} = m_i - m_j , \quad i < j, \; i=1,..,6, \; j=2,...,7.$$

are compared with LSD from (A7.3) at a particular significance level. From this table it can be decided which means, m_i, form subsets, in which the elements do not differ significantly from one another. These subsets may have nonzero intersection.

A7.4.2 LINK-WALLACE MULTIPLE COMPARISONS (Sachs, 1984).

Using the critical value of $K_{n,k;\alpha}$ (from section A7.2) pairwise comparisons of all the compartment means are done with the mean difference $D_{LW;\alpha}$. They are

significant at confidence level $(1-\alpha)\times 100\%$ if they exceed

$$D_{LW;\alpha} = \frac{K_{n,k;\alpha} \sum R_i}{n} \qquad \ldots(A7.4)$$

Here also the compartment (treatment) means are arranged just as for the Fisher LSD test above, but instead of using LSD_α, $D_{LW;\alpha}$ is used. The differences, d_{ij} $(=m_i-m_j)$, have a similar interpretation as above.

A7.4.3 WILCOXON & WILCOX TEST (Sachs, 1984).

This corresponds to the Friedman test. Again k compartments (treatments) with n observation time instants (replications) each are compared. Every compartment (treatment) is assigned a rank from 1 to k, so that n rank orders result. The ranks of individual samples are added; their differences are compared with the value of the critical difference. If the tabulated critical value is exceeded (or attained) then the compartment samples (treatments) come from different statistical populations (or distributions). If the computed difference falls below the tabulated $D_{WW;\alpha}$, the difference can still be regarded as accidental.

Here, the column rank sums are first ordered by magnitude

$$r_1 > r_2 > \ldots > r_7$$

and then a table similar to the one for the Fisher-LSD test is formed with r_i in place of m_i. The differences, d_{ij}, are now

$$d_{ij} = r_i - r_j \quad , \quad i < j, \; i=1,..,6, \; j=2,...,7.$$

A7.5 TESTS FOR DEPARTURE FROM GAUSSIANITY.

Some statistical tests to check Gaussianity will be described now. Various tests are available. Here tests for skewness and kurtosis are used.

Test of Skewness. Let m_3 and m_2 denote, respectively, the third and second central moments of the sample. The coefficient of skewness, g_1, is

$$g_1 = m_3/(m_2)^{3/2} \quad . \qquad \ldots(A7.5)$$

The sampling distribution of g_1 is approximately Gaussian (sample size, N > 150) with zero mean and variance 6/N, if the sample is Gaussian (Patel & Read, 1982). For N between 25 and 200 critical values for g_1 have been tabulated for tests at 95% and 99% confidence levels. (See Pearson & Hartley, 1966)

Test for Kurtosis. Let m_4 denote the fourth central moment of the sample. Then the kurtosis of the sample, g_2, is given by

$$g_2 = m_4/m_2^2 - 3 \qquad \ldots(A7.6)$$

For a normal sample, if the size is very large, g_2 has a Gaussian sampling distribution with zero mean and variance 24/N (Patel & Read, 1982). The critical values of g_2 are available in tables. (See Pearson & Hartley, 1966)

A7.6 CHOICE OF PARAMETRIC VS. NONPARAMETRIC TESTS

If statistical tests are evaluated in terms of

power and robustness (power being "sensitivity to change in the specific factors tested" and robustness being "insensitivity to changes, of a magnitude likely to occur in practice, in extraneous factors".), parametric tests (e.g. classical ANOVA) are derived to be powerful for an assumed specific probability distribution. But, unless their assumptions are met, there is a yielding in robustness and such tests may not even be valid. Thus robustness is of great concern. Unlike these, nonparametric tests (e.g. Friedman test) are inherently robust because they are based on very general assumptions. Where comparison studies have been made nonparametric tests are frequently almost as powerful as their parametric counterparts, especially for small samples (Gibbons, 1985), and therefore may be considered more desirable whenever there is any doubt about assumptions. For this reason decisions made from nonparametric tests have been given preference in this study. A summary of the results of all the tests is given in Table 23

Table 23

SUMMARY OF STATISTICAL TESTS

WLS = Weighted Least Squares estimation
-2LL = -2 Ln Likelihood estimation

I. CLASSICAL ANALYSIS OF VARIANCE
(Values of F)

	WLS	-2LL
DISCRETE:		
1st Moments	4.61**	4.52**
2nd Moments	11.43**	11.35**
CONTINUOUS:		
1st Moments	4.77**	4.89**
2nd Moments	6.86**	6.86**

II. LINK-WALLACE ANALYSIS OF VARIANCE
(Values of K)

	WLS	-2LL
DISCRETE:		
1st Moments	1.016**	1.008**
2nd Moments	1.817**	1.814**
CONTINUOUS:		
1st Moments	1.169**	1.199**
2nd Moments	1.162**	1.165**

III. THE FRIEDMAN TEST
(Values of χ_R^2)

	WLS	-2LL
DISCRETE:		
1st Moments	25.68**	25.68**
2nd Moments	45.33**	45.33**
CONTINUOUS:		
1st Moments	26.74**	26.60**
2nd Moments	25.12**	25.12**

* denotes significance at $\alpha = 0.05$

** denotes significance at $\alpha = 0.01$

APPENDIX 8

A BRIEF REVIEW OF THE IMMUNE SYSTEM

This appendix briefly reviews the immune system. As mentioned in section 1.2 there are different types of immunity. These include those that are non-specific (or innate) and those that are specific (or acquired) (Guyton, 1976). Both of these types of immunity may be either humoral or cellular. There is a continuous interaction between all these different mechanisms as shown in figure 11, where the broken lines indicate

reactions influenced by T-cells.

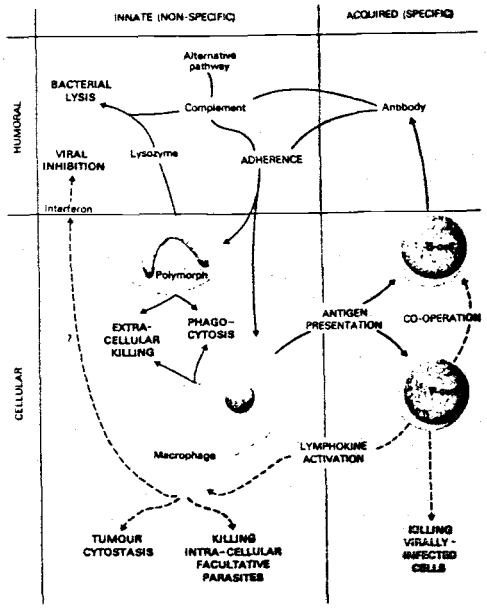

Figure 11. Simplified scheme showing interactions between natural and acquired immunities (Roitt, 1980)

This appendix will be concerned only with acquired immunity, the existence of all the other defense mechanisms and their interactions being assumed. These may to a degree contribute to the "noise" due to uncertainty in the models presented in this thesis.

A8.1 LYMPHOCYTES.

Acquired immunity is based on the existence and proper functioning of certain cells which are called lymphocytes and are a type of leukocytes (white blood cells) (Guyton,1976). There are two types of lymphocytes which have been named T-cells and B-cells. In mammals both cells originate in the bone marrow as stem cells and are processed in different places. The stem cells which are processed in the thymus are called T-cells or T-lymphocytes, while those processed independent of the thymus are called B-cells or B-lymphocytes. It has been difficult to pinpoint this thymus-independent processing region(s) and different authors have speculated about different areas of gut associated lymphoid tissue. In the fetus it is the liver and in the adults it may be bone marrow (McConnell, Munro, & Waldman, 1981) and/or spleen (Sprent, 1977) or hematopoietic tissue (see Kincade & Moore, 1977, pp 134-136 for details). For a recent view on more detailed classification of lymphocytes see Petrov (1984).

A8.2 ORGANS OF THE IMMUNE SYSTEM.

The immune system functions in concert with the

reticulo-endothelial system and is distributed throughout the body in the form of discrete, strategically placed lymphoid organs, some of which comprise the primary lymphoid tissue and the rest the secondary lymphoid tissue. Primary lymphoid tissue is that where lymphocytes are produced and processed (at a basic level), namely, bone marrow and thymus. The rest of the lymphoid tissue (where lymphocytes are further processed) is called secondary, namely, spleen, lymph nodes (LN), gut associated lymphoid tissue (GALT), bronchial associated lymphoid tissue (BALT), etc. The distribution of the lymphoid system is shown in figure 12.

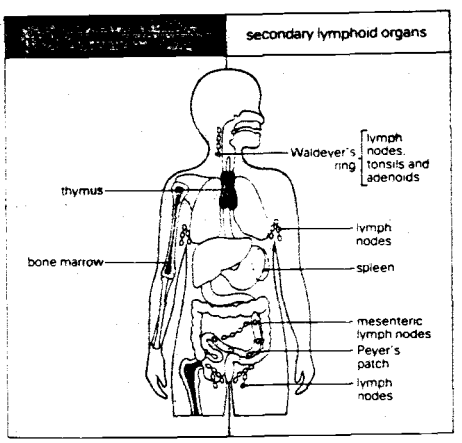

Figure 12. The immune system
(Roitt, 1985)

For details of the structure and functions of the individual organs see any standard textbook on anatomy, physiology, or histology.

A8.3 THE IMMUNE RESPONSE.

The immune response (i.e. the reaction to foreign invasion) can be divided into three phases:

i) Initial phase --- the events between entry of antigen and its presentation to antigen receptors on the lymphocytes,

ii) Central phase --- the interactions between different subpopulations of lymphocytes, and

iii) Effector phase --- when the antigen is the target of destruction.

These phases are illustrated in figure 13. Virgin lymphocytes become activated or sensitized when they come into contact with an antigen and differentiate further into either memory cells or effector cells (T- or B- depending on what they were as virgin

lymphocytes).

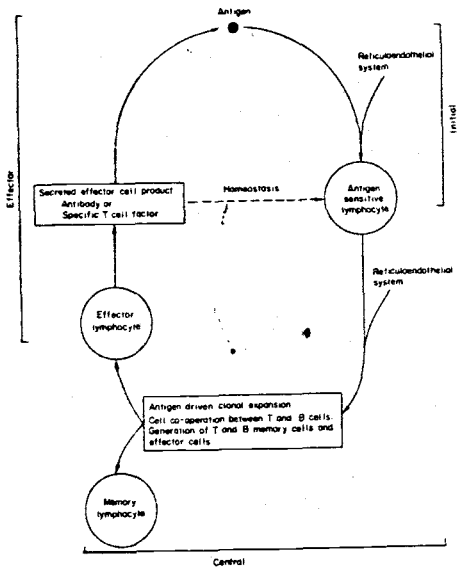

Figure 13. The three phases of an immune response (McConnell, et.al., 1981).

A8.4 EFFECTOR LYMPHOCYTES.

T-lymphocytes have been found to respond to four different types of reactions (Roitt,1980):

i) mixed lymphocyte reaction (T-helper or T_h),

ii) allograft cytotoxicity (T-cytotoxic or T_k),

iii) carrier-specific hyperactivity (T_d), and

iv) a potential suppressor function (T-suppressor or T_s).

The T-helper cells, when they recognize and respond to an antigen, help B-lymphocytes specific for the antigen to develop into antibody-forming cells. The hypersensitivity reaction is initiated by an antigen, which may be associated with or processed by a macrophage, combining with the receptors on the surface of appropriate T-memory cells. This transforms into a large blast cell and undergoes mitosis. A proportion of these stimulated T-cells release a number of soluble factors (including lymphokines) which mediate the ensuing hypersensitivity response, while some others develop cytotoxic powers. A class of T-cells can suppress the activity of B-lymphocytes and also other T-lymphocytes.

The functioning of T-cells depends probably on receptors bound to their membranes. In B-cells the existence of membrane-bound antibodies is known and they are also released into the blood. Thus B-lymphocytes are responsible for the humoral immune response and the T-lymphocytes for a significant part of the cell-mediated immune response.

Although many believe that there is no

T-independent humoral response in humans, others assume two types of B-cells, one of which depends on T-cell cooperation for activation and the other does not (Roitt, 1980). Both of these types of cells release antibodies or immunoglobulins (IgG, IgA, IgM, IgD, and IgE) having different properties (proportions in the body, molecular weights, number of basic tetra-peptide units, valency for antigen-binding, etc). For details see Roitt (1980), Fudenberg, et. al. (1978), Benacerraf & Unanue (1979), or any other standard book on the fundamentals of immunology. Some books dealing entirely with the structure and function of the lymphoid system are: Rusznyak et.al. (1967), Yoffey & Courtice (1970), Elves (1972), Trnka & Cahill (1980), and Gowans (1980). A concise summary of white blood cells is given in Boggs & Winkelstein (1984).

A8.5 PRIMARY AND SECONDARY RESPONSES.

On first exposure to an antigen the events mentioned in figure 13 above occur. This is called the primary immune response. On subsequent exposure to the same antigen the response involves memory cells and is much quicker and amplified. This is refered to as the

secondary response.

These responses apply to both B- and T-cells. It is important that the responses are based on the selective, expansion and suppression of different clones of antigen-specific T- and B-lymphocytes. The main factors currently thought to modulate the immune response are summarized in figure 14.

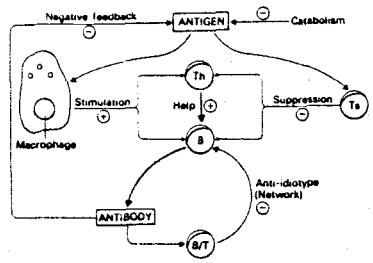

Figure 14. Regulation of the immune response. T_h = T-helper cell; T_s = T-suppressor cell. (Roitt, 1980)

APPENDIX 9

MATHEMATICAL MODELS IN IMMUNOLOGY & BIOMEDICINE.

Models of some aspects of the immune system are presented below.

A9.1 THE HUMORAL IMMUNE RESPONSE.

Historically, Hege & Cole (1966) presented the first relevant model relating the changes in circulating antibody with the number of antibody-producing cells. The models that have been published since then can be divided into the following categories. Most of the models below are discussed in detail in Merrill (1980), and Mohler, Bruni, & Gandolfi (1980). Some of them are more recent. Most models are based on clonal selection theory.

1. Discrete Affinity

The basic B-cell model (Mohler et.al., 1980) is:

$$\dot{\underline{x}} = A\underline{x} + (\underline{b}_1\nu_1 + \underline{b}_2\nu_2)x_1 + \underline{b}_3\nu_3 x_3 + \underline{c}_4 u_1 + \underline{c}_5 u_2$$

$$\underline{x} = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \\ x_5 \end{bmatrix}, \quad b_1 = \begin{bmatrix} \alpha \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \quad b_2 = \begin{bmatrix} 0 \\ 2\alpha \\ 0 \\ 0 \\ 0 \end{bmatrix}, \quad b_3 = \begin{bmatrix} 0 \\ 0 \\ -c \\ c \\ -Nc \end{bmatrix}, \quad c_4 = \begin{bmatrix} 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \quad c_5 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{bmatrix}$$

$$A = \begin{bmatrix} -1/\tau_1 & 0 & 0 & 0 & 0 \\ 0 & -1/\tau_2 & 0 & 0 & 0 \\ \alpha'' & \alpha' & -1/\tau_3 & c & 0 \\ 0 & 0 & 0 & -(c+1/\tau_4) & 0 \\ 0 & 0 & 0 & Nc & -1/\tau_5 \end{bmatrix}$$

where

x_1 = population concentration of ICC (immunocompetent cells) with surface receptors having association constant or affinity k for Ag,

x_2 = pop. conc. of short-lived plasma cells,

x_3 = pop. conc. of free "Ab-sites",

x_4 = pop. conc. of immune complexes,

x_5 = Ag. conc.

τ_i = mean lifetime of x_i, i = 1,...,5

$u_1(t)$ = rate of generation of new ICC (from bone marrow),

$\nu_1 = p_s(1 - 2p_d)$, $\nu_2 = kx_5$, $\nu_3 = p_s p_d$,

p_s = probability that Ag stimulates a cell,

p_d = probability that an ICC differentiates into a plasma cell,

α' = plasma cell Ab-production rate,

α'' = ICC Ab-production rate,

c, kc = dissociation, association rate coefficients, respectively, of immune complexes

$u_2(t)$ = inoculation rate of Ag.

$\underline{u} \in \mathbb{R}^2$ function as additive control, $\underline{\nu} \in \mathbb{R}^3$ as multiplicative control. Existence and uniqueness of the solution of this bilinear structure and its reachability properties have been studied. Mohler & Hsu (1978) followed with a compartmental model using blood, spleen, lymph, and lymph nodes as compartments and each compartment with four states as ICC, plasma cells, Ab, and Ag. Other similar models include those of Bell (1970, 1971a, 1971b). Waite & Hyer (1986) have developed a model for T-cell independent response incorporating persistent signalling mechanism and auto-catalysis (auto-regulation of the response by B-cells) for termination of the response.

2. Continuous Affinity (Bruni, et.al., 1975a, 1975b, 1978)

Here instead of assuming discrete constant values for the Ag-receptor association constant it is assumed to have any value in some interval (k_1, k_2).

3. Network Models (Jerne, 1973, 1976; Richter, 1974, 1978; Hoffmann, 1975, 1978)

Jerne introduced the concept of interclonal interaction to take into account the antigenic nature of antibody molecules. Richter developed the mathematical model based on the following possible sequence of reactions (adapted from Merrill, 1980)

1. low-level response: $Ag \xrightarrow{\text{stimulates}} Ab_1$
   Ab_1 levels below threshold for Ab_2 stimulation.
2. low-zone tolerance: $Ag \xrightarrow{\text{stim}} Ab_1 \xrightarrow{\text{stim}} Ab_2$
   Ab_2 inhibits Ab_1-Ag combination.
3. normal response: $Ag \xrightarrow{\text{stim}} Ab_1 \xrightarrow{\text{stim}} Ab_2 \xrightarrow{\text{stim}} Ab_3$
   Ab_3 eliminates Ab_2 inhibition of Ab_1-Ag combination.
4. high-zone tolerance: $Ag \xrightarrow{\text{stim}} Ab_1 \xrightarrow{\text{stim}} Ab_2 \xrightarrow{\text{stim}} Ab_3 \xrightarrow{\text{stim}} Ab_4$
   Ab_4 eliminates Ab_3, response appears as in low-zone tolerance.

Hoffmann expanded the model to include T-suppressor cells which produce a monovalent "blocking" substance instead of antibodies (Ab's) and are faster reacting than B-cells. Recently Fey & Eichmann (1985) described suppressive regulation between polyclonally activated normal and immune T-cells using this approach.

4. Threshold models

Waltman & Butz (1977) consider delays in the response by generating delays from threshold conditions (as in epidemic models).

5. T-B cellular/multicellular interactions

Mohler et.al. (1978), Marchuk (1979a), and Asachenkov & Marchuk (1979) have described some interactions. A recent model is that of Kaufman, Urbain, & Thomas (1985) who first use Boolean functions to develop a model of Ag, B, T_h, T_s interaction and then translate it to homologous continuous differential equations and replace logic variables by sigmoidal functions.

6. Stochastic models

Jilek (1971a, 1971b) and Jilek & Sterzl (1971) base their models on the probability of contact between a cell (through appropriate Ab receptor) and an antigen (Ag).

7. Other models

Dibrov et.al. (1977a, 1977b) and Merrill (1977, 1980) have described the cyclic behavior of the response. Gunther & Hoffmann (1982) have discussed the IgM-IgG switchover.

A9.2 CYTOTOXICITY

There have been some attempts at modeling the cell-mediated immune response also. Two types of cytotoxicity are described below.

A9.2.1 CYTOTOXICITY DUE TO T-CELLS.

Garay & Lefever (1978) and Lefever (1980) have tried to model the cytotoxic T-lymphocyte (CTL) activity on tumor cells. They view the response to occur in the following chronological order:

i) Hidden cancer: a clone of cells arises from a transformed cell and multiplies freely. Presence of this proliferation is not yet recognized by the body.

ii) Recognition: T-cells detect the antigenic neoplastic cells and come back to lymphoid organs to induce clone proliferation of the relevant effector cells,

iii) Immune response: the effector T-cells reach tumor by vascular system (i.e. migration) and may trigger a complex series of phenomena (e.g. activation and infiltration of tumor by other effector cells), and

iv) Suppression: Production of T -cells and

their complexes with other mechanisms form blocking agents to inhibit the immune response. The events in iii) are illustrated in figure 15.

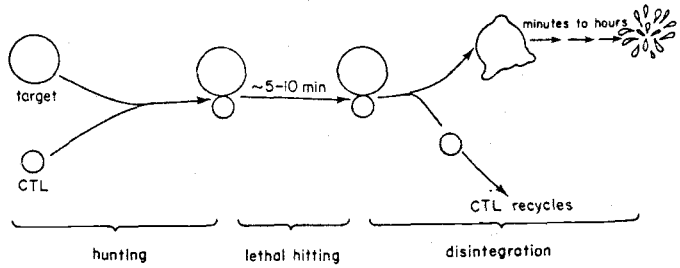

Figure 15. The steps in a cytotoxic T lymphocyte (CTL) attack on a target cell. (Macken & Perelson, 1985)

The reaction is thought to have Michaelis-Menten kinetics (similar to that of enzymes) and it is assumed to occur in a multicompartment system as follows:

$$X + Y \rightleftharpoons XY \longrightarrow Y + P$$

The equations derived are as follows:

$$\frac{\partial x}{\partial t} = (1 - \theta x)x - \beta xy + a^2 \frac{\partial^2 x}{\partial r^2}$$

$$\frac{\partial y}{\partial t} = (z - xy) + \mu \frac{\partial^2 y}{\partial r^2}$$

$$\frac{\partial z}{\partial t} = (xy - z)$$

$$x = k_1 X/k_2, \quad y = Y/E_t, \quad z = Z/E_t,$$

$\beta = k_1 E_t/\lambda$, $\theta = k_2/k_1 N$, $\rho = k_2/\lambda$, $\mu = D/\lambda$,

$E_t = Y + Z$,

k_1, k_2 constant,

X, Y, Z = # cells of diameter a in a box,

(X = Target cells, Y = Effector cells, and

Z = Complex X-Y),

N = max # cells the tumor mass could contain,

λ = replication constant,

D = Diffusion coefficient of free effector cells (which are the only ones which may diffuse).

Steady-state properties of this model have been investigated, conditions of tumor rejection obtained and checked against experimental data. Perelson and Macken (1984) have also presented a deterministic model of the same phenomena. Stochastic models of cell-mediated cytotoxicity include those for lethal-hitting, target cell disintegration, and CTL frequency (Perelson & Macken, 1984; Macken & Perelson, 1985).

A9.2.2 CYTOTOXICITY DUE TO NATURAL KILLER CELLS.

Merrill (1981a, 1983) suggests the following model for natural killer (NK) cells, which are

considered to be "large granular lymphocytes". It is assumed that no T-cells are present and there is no Ab-dependent cytotoxicity. The following assumptions were made:

i) NK cells appear at a constant rate s from there source in the bone marrow,

ii) maturation rate of pre-NK to NK cells is an increasing function of interferon concentration,

iii) NK cells produce interferon when on contact with a tumor, and interferon concentration is spatially nearly homogeneous,

iv) Tumor growth is affected by interferon present and the rate of cytotoxicity of NK cells, and

v) Other mechanisms do not change.

The model presented is:

$$\frac{dx_1}{dt} = s_1 - k_1 x_1 x_3 - k_2 x_1$$

$$\frac{dx_2}{dt} = k_1 x_1 x_3 - k_3 x_2 + k_2' x_1$$

$$\frac{dx_3}{dt} = s_2 - k_4 x_3 + g_1(x_2, x_4) - k_1' x_1 x_3 - g_2(x_3, x_4)$$

$$\frac{dx_4}{dt} = g(x_4, x_3) - g_3(x_2, x_3, x_4)$$

$$s_1, s_2, k_1, k_1', k_2, k_2', k_3, k_4 \geqslant 0;$$

$$\xi_1(0, x_4) = \xi_1(x_2, 0) = 0;$$

$$\xi_2(0, x_4) = \xi_2(x_3, 0) = 0;$$

$$\frac{\partial \xi_1}{\partial x_2} > 0; \qquad \frac{\partial \xi_1}{\partial x_4} > 0;$$

$$\gamma(x_3, 0) = \xi_3(0, x_3, x_4) = \xi_3(x_2, x_3, 0) = 0;$$

$$\frac{\partial \gamma}{\partial x_3} \leqslant 0; \quad \frac{\partial \xi_3}{\partial x_2} > 0; \text{ and } \frac{\partial \xi_3}{\partial x_4} > 0;$$

$$x_1(0), x_2(0), x_3(0), x_4(0) \geqslant 0$$

x_1 = Pre-NK cells

x_2 = "Mature" NK cells

x_3 = Interferon

x_4 = Tumor cells

Existence, uniqueness, and stability of the solutions of this system of equations have been studied and the conditions for elimination of the tumor found.

A9.3 INTERACTION OF TUMOR CELLS AND CELLS OF THE IMMUNE SYSTEM.

This interaction has already been mentioned under

cytotoxicity (Garay & Lefever, 1978; Lefever & Garay, 1978; Lefever, 1980; Merrill, 1981a; Merrill, 1983). Tumor escape has been discussed by Grossmann & Berke (1980), De Boer & Hogeweg (1985) and Michelson (1986) and the role of macrophage-T cell interaction by De Boer et.al. (1985). Many papers do not specify the cells of the immune system that are involved and in this way implicate T-cells, NK cells, macrophages, and any as yet unidentified cell population in defense against the tumor (DeLisi & Rescigno, 1977; Rescigno & DeLisi, 1977; Merrill, 1979; Albert, Freedman & Perelson, 1980).

A9.4 MODELS OF OTHER IMMUNE PHENOMENA.

Models of diverse immune phenomena have been proposed. A small random sampling follows. Fowler (1981) discusses delays in immune responses, Klein, Sterzl, & Dolezal (1983) B-cell differentiation, and Klein, Hraba, & Dolezal (1983) use tolerance to investigate B-cell replacement. Merrill (1981b) has a model for control and activation of the complement system. Pilz & Tautu (1984) have studied instabilities in the stem cell system, while Blumenson (1975) and McFarland & Van der Vaart (1985) worked on the

granulocyte system, and Rittgen (1983) on the hematopoietic system. Lauffenberger & Kennedy (1983) have presented a model of distributed inflammation.

A9.5 DISEASE.

In pathological states there is a deviation in immune functioning (either heightening or depression). Thus it is important to consider disease models together with immune models. Models of carcinogenesis have been reviewed by Whittemore & Keller (1978) and those in oncology by Whittemore (1977) and Newton (1980). Many models of disease (e.g. hepatic lesions and viral diseases), epidemiology, and estimation of disease state are given in Marchuk (1978), Marchuk (1983), and Marchuk & Belykh (1983).

APPENDIX 10

GLOSSARY OF TERMS USED IN IMMUNOLOGY

This glossary covers most of the immunological terms used in this thesis. Some of the terms may have more than one definition; the definitions given here are the ones pertinent to this project. For more details see any textbook on fundamentals of immunology or any medical dictionary (Some of the definitions here are from the Dorland's Illustrated Medical Dictionary).

ANTIBODY (Ab) --- An immunoglobulin molecule that has a specific amino acid sequence by virtue of which it interacts only with the antigen which induced its synthesis. (For details see Roitt, 1980)

ANTIGEN (Ag) --- Any substance which is capable, under appropriate conditions, of inducing an adaptive immune response. Ag's may be soluble, such as toxins, or particulate, e.g. bacteria.

B-CELL --- An avian bursa-derived cell. By analogy a non-thymus derived lymphocyte in non-avian species.

B-LYMPHOCYTE --- B-cell.

BALT --- Bronchial Associated Lymphoid Tissue.

BLAST CELLS --- A large lymphocyte or other immunocyte containing a nucleus with loosely packed chromatin, a large nucleolus, and a large amount of cytoplasm with numerous polyribosomes.

BM --- Bone Marrow.

CAPILLARY --- The minute vessel connecting an arteriole and a venule. Its walls act as semi-permeable membranes for the interchange of blood and tissue fluid.

CARCINOGENESIS --- Production of carcinoma (malignant new growth).

CELL MEDIATED IMMUNITY --- Acquired immunity in which the participation of T lymphocytes and macrophages is predominant.

CLN --- Coeliac Lymph Node.

CLONAL SELECTION THEORY --- The theory of Ab synthesis proposed by Burnet which predicts that the individual carries a complement of clones of lymphoid cells capable of reacting with all possible determinants. (See Roitt, 1980).

CMI --- Cell Mediated Immunity.

COMPLEMENT --- A complex series of enzymatic proteins occuring in normal serum that interact to combine with Ag-Ab complex, producing lysis when the Ag is an intact cell. It comprises 11 discrete proteins, or 9 functioning components symbolized C1 through C9, with C1 being divided into subcomponents C1q, C1r, and C1s. (See Roitt, 1980)

CYTOTOXICITY --- The quality of being capable of producing a specific toxic action upon cells of special organs.

DISEASE --- A definite morbid process having a characteristic train of symptoms; may affect whole or

part of the body; its etiology, pathology, and prognosis may be known or unknown.

ECOTAXOPATHY --- An abnormality in the migration pattern of lymphocytes.

EFFECTOR CELL --- A cell mounting an immune response as a reaction to an Ag; usually a T-cell capable of mediating cytotoxicity.

ERYTHROCYTE --- Red blood cell (RBC).

GALT --- Gut Associated Lymphoid Tissue.

HEMATOPOIETIC --- Pertaining to or affecting the formation of blood cells.

HEMODYNAMICS --- Study of the movements of the blood and of the forces concerned therein.

HEV --- High Endothelial Venules.

HUMORAL IMMUNITY --- Acquired immunity in which the role of circulating Ab's (from B lymphocytes) is predominant.

HYPERSENSITIVITY (REACTION) --- A state of altered reactivity in which the body reacts with an exaggerated response to an Ag. There are 4 types of such a reaction. (See Roitt, 1980)

IMMUNE SYSTEM --- The system that defends the body against pathogens.

IMMUNOLOGY --- The study of an organism's response to an antigenic challenge, recognition of self from non-self, and all biological, serological, and physico-chemical aspects of the immune phenomena.

ISOANTIGEN --- An Ag that exists in alternative forms in a species and can elicit a reaction in a member of the same species.

LABEL --- A radioactive isotope introduced into tissue to identify the role of the normal element in metabolism.

LAF --- Lymphocyte Activating Factor.

LCF --- Lymphocyte Chemotactic Factor.

LEUKOCYTE --- White blood cell (WBC).

LH --- Left Heart.

LIF --- Leukocyte Inhibition Factor.

LN --- Lymph Node(s).

LT --- Lymphotoxin. Here has also been used for Lymphoid Tissue. The use is clear from the context.

Ly SYSTEM --- The system of differentiating Ag's present on thymocytes and peripheral T-cells.

LYMPH NODE --- Lymphoid tissue involved in secondary processing of lymphocytes.

LYMPHOBLAST --- The immature, nucleolated precursor of the mature lymphocyte.

LYMPHOCYTE --- A mononuclear leukocyte 7-20 μ in diameter (in humans), with a deeply staining nucleus; chiefly a product of lymphoid tissue.

LYMPHOCYTOPENIA --- A reduction in the number of

circulating lymphocytes.

LYMPHOCYTOSIS --- An excess in the number of circulating lymphocytes.

LYMPHOID SYSTEM --- Lymphatic vessels and lymphoid tissue considered collectively.

LYMPHOKINE --- A general term for soluble protein mediators (other than Ab) released by sensitized lymphocytes on contact with Ag and believed to play a role in macrophage activation, lymphocyte transformation, and CMI.

MACROPHAGE --- A phagocytic mononuclear cell that derives from bone marrow monocytes and subserves accesory roles in CMI.

MAF --- Macrophage Activating Factor. Also an acronym for Macrophage Aggregation Factor.

MCF --- Macrophage Chamotactic Factor.

MIF --- Macrophage migration inhibitory Factor.

MIFIF --- MIF Inhibition Factor.

MIGRATION --- Movement of WBC through vessel walls.

MLN --- Mesenteric Lymph Node.

MONOCYTE --- A mononuclear phagocytic leukocyte, 13-15 μ in diameter.

MONONUCLEAR CELL --- A cell having a single nucleus.

MUCOSA --- A mucous membrane.

OEL --- Other Efferent Lymphatics.

ONCOLOGY --- The study of tumors.

OT --- Other Tissues.

PHAGOCYTE --- Any cell that ingests microorganisms or other cells and foreign particles.

PHYSIOLOGY --- The science which treats of the functions of the living organism and its parts, and the physical and chemical factors and processes involved.

PLASMA CELL --- A fully differentiated Ab-synthesizing cell which is derived from a B lymphocyte.

POLYMORPHS --- Polymorphonuclear leukocytes.

POLYMORPHONUCLEAR LEUKOCYTE --- A WBC having a nucleus deeply lobed or so divided that it appears to be multiple.

PP --- Peyer's Patches.

RBC --- Red Blood Cell.

RECEPTORS --- A specific chemical grouping on the surface of an immunocompetent cell with the capability of combing specifically with an Ag.

RECIRCULATING LYMPHOCYTE ---A lymphocyte that circulates from blood to lymph to blood over and over again. Such cells are long-lived, most likely memory cells, and are primarily T-cells.

RECIRCULATING LYMPHOCYTE POOL --- The portion of the lymphocyte population that recirculates.

RED BLOOD CELL --- The blood cell involved in oxygen transport; has red color because of hemoglobin.

RH --- Right Heart.

SCLN --- Subcutaneous Lymph Node(s).

STEM CELLS --- Generalized mother cells whose descendents specialize, often in different directions.

T-CELL --- T lymphocyte.

T LYMPHOCYTE --- A thymus derived cell that participates in a variety of CMI reactions.

TD --- Thoracic Duct.

TDL --- Thoracic Duct Lymphocyte(s).

TMIF --- Tumor cell Migration Inhibition Factor.

TRACEE --- The element that the tracer identifies.

TRACER --- Label.

TRAPPING --- Lymphocyte retention in an antigenically stimulated LN.

VASCULAR ENDOTHELIUM --- Epithelial cells lining the cavities of blood vessels.

WBC --- White Blood Cell.

WHITE BLOOD CELL --- One of the several types of cells involved in the defense of the body and having characteristic morphological and histological features.