

ABSTRACT

WHITAKER, SHREE YVONNE. A Biologically-Based Controlled Growth and Differentiation Model Using Delay Differential Equations: Development, Applications and Stability Analysis. (Under the direction of Hien T. Tran.)

This work investigates the development, applications and stability analysis of a biologically-based dose-response model for developmental toxicology. The biologically-based controlled growth and differentiation model is based on a model originally developed by Leroux et al. (1996). The original model had two basic states; precursor cells and differentiated cells with both states subject to a linear birth-death process. The research discussed in this dissertation describes the development of a mathematical model that is both biologically- and statistically-based. The model is developed with a highly controlled birth and death process for precursor cells. This model limits the number of replications allowed in the development of a tissue or organ and more closely reflects the presence of a true stem cell population. The mathematical formulation of the Leroux et al. (1996) model was derived from a partial differential equation for the generating function that limits further expansion into more realistic models of mammalian development. The same formulae for the probability of a defect (a system of ordinary differential equations) can be derived through the Kolmogorov forward equations due to the nature of this Markov process. This modified approach is easily amenable to the expansion of more complicated models of the developmental process. Comparisons between the Leroux et al. (1996) model and the controlled growth and differentiation (CGD) model are also discussed.

The versatility of the CGD model is highlighted through a discussion of two general applications. The normal developmental process of spermatocytogenesis is investigated as the first application. Time delays are introduced into the system to more accurately mimic the development of male germ cells. As the second application, the spermatocytogenesis model is then altered to demonstrate a modeling strategy for hormesis. Asymptotic stability is investigated using the system of delay differential equations for spermatocytogenesis. The direct Lyapunov method for linear differential equations without delay is modified to establish delay-dependent stability conditions for delay differential equations with multiple delays. The stability conditions are expressed in terms of the existence of a positive definite solution to the Riccati matrix equations. Numerical simulations further verify the stability conditions.

**A BIOLOGICALLY-BASED CONTROLLED GROWTH AND
DIFFERENTIATION MODEL USING DELAY DIFFERENTIAL EQUATIONS:
DEVELOPMENT, APPLICATIONS AND STABILITY ANALYSIS**

by

SHREE YVONNE WHITAKER

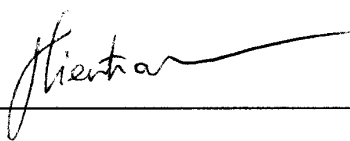
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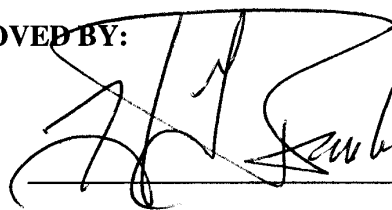
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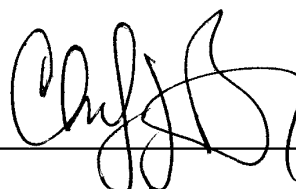
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Biography

Shree Yvonne Whitaker was born February 7, 1972 in Atlanta, Georgia. She is the third of four daughters of Mr. and Mrs. John T. Whitaker. She attended public schools in DeKalb County and in June 1990, graduated as the valedictorian of Ronald E. McNair Senior High School. Shree completed the BS and MS degrees in Mathematics at Clark Atlanta University on a five-year scholarship program funded by the Office of Naval Research (ONR PRISM-D Program). In the fall of 1995, she started North Carolina State University as a Department of Defense Fellow. Through dedication and perseverance she earned the doctorate of philosophy in Applied Mathematics, Computational Mathematics Concentration in December 2000.

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“I am because we are.”

I am grateful to so many for so much. This accomplishment has definitely been a team effort. I appreciate the guidance and “open door” policy of my advisor, Dr. Hien Tran. I am thankful for his high standards and patience. They are trademarks that I constantly strive to attain for myself. The positive reinforcement of Dr. Christopher Portier (Laboratory of Computational Biology and Risk Analysis, National Institute of Environmental Health Sciences) has done so much for my self-image as a young mathematician in the research field. His distinct up-beat personality has made training with him a scientific adventure and a pleasure. I admire his ability to “think outside the box” and to solve fascinating biomedical problems using mathematics as a tool. I would also like to thank Dr. Robert E. Chapin (Laboratory of Developmental and Reproductive Toxicology, National Institute of Environmental Health Sciences) for taking the time to explain the fundamentals of biology to me over and over again! I sincerely appreciate all the time and effort from him and his laboratory. I would also like to express my deepest gratitude to Dr. H. Thomas Banks. His everlasting enthusiasm for mathematics and North Carolina State University has been a continuous motivation for me! I would like to thank him for establishing the Center for Research in Scientific Computations and training students to be educators as well as interdisciplinary researchers. I also greatly appreciate his professional and financial assistance. All of these individuals have constantly provided me with guidance,

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Chapter 1: Background and Overview

The biological process of a developing tissue or organ in mammals is a complex activity. Systems of cells behave in various ways to reach the goal of forming a properly functioning tissue or organ. For normal development of a mammalian tissue or organ, the cell population must be within a specified interval at a critical time of development. A small deviation in the developmental process can result in malformation. The probability of malformation surfaces either when there are not enough cells produced to carry out a specific function or when a certain population of cells over-produces. The controlled growth and differentiation (CGD) model is intended to be able to predict the probability of malformation for a tissue or organ. The proposed model is based on the mathematical model developed by Leroux et al.; however, the CGD model has been designed to be more versatile, adaptable and thus more powerful than the original model. Due to its structure, the CGD model can also be used to explore intermediate stages of normal or abnormal development.

For a well developed presentation of the research highlighted by this dissertation a brief background of mathematical modeling is discussed in Section 1.1. This focus is then reduced to the subset of biological modeling in Section 1.2. Section 1.3 discusses

the use of delay differential equations in modeling. The chapter is concluded with a statement of the problem and an outline of the dissertation in Section 1.4.

1.1 Mathematical Modeling

Mathematical modeling has been used as a tool to understand various dynamical processes for centuries. However, the marriage of computer technology and mathematical modeling has recently allowed researchers to investigate problems that were once described as impossible and frivolous tasks. The ability to study system dynamics with elegance, speed and accuracy has been a great benefit for the advancement of scientific research.

While mathematics is unboubtedly a powerful tool, its power must be handled with great care and the highest respect when modeling. To use models properly requires not only an understanding of mathematics, but also a fundamental knowledge of the dynamic process under study. Once a mathematical model is developed for a particular system, this does not constitute the end of the problem. In fact, modeling can be thought of as an iterative process. After a model is constructed, it must be tested (preferably against real experimental data) for validity. This step is followed by continuous refinement until the mathematical model mimics the dynamical process as accurately as computationally possible.

Although computational mathematics has several advantages, the disadvantages must also be acknowledged. Nature (and other mechanical processes) is very complex

and often involves several highly coupled dynamics all happening at once. Thus when building a mathematical model, the investigator must be careful not to construct a model that can not be understood at each intermediate step. To this end, assumptions in the development of a model may be physical, biological or purely mathematical [1]. It is quite common for researchers to impose restrictions on the model that compromises the true dynamics of the process. Otherwise, the model will be intractable and no further insight into the phenomena under scrutiny will be gained.

1.2 Biological Modeling

One major application of mathematics is its use to model and understand biological processes. For example, development of the human body is a fascinating, yet complicated, series of events. From the moment of conception until birth, the cells of the embryo are programmed to carry out a specific task. Various “checkpoints” and regulators are in place to monitor and control the developmental process. However, there are instances when a cell or group of cells will stray from their prescribed schedules. In some cases, neighboring cells are able to compensate for the blunder. But in other cases, the problem cannot be corrected for various reasons. In the event a system of cells does not function according to certain biological specifications, a malformation may occur. Such malformations may include a specific cell population with reduced or increased numbers of cells or a cell population where the cells simply do not function properly.

There has been considerable research into the mechanistic basis through which environmental exposures can initiate and promote disease processes. Much of this research has focused on the molecular and biochemical basis describing the interaction of chemical and physical agents with healthy tissue. Most environmental health risk assessments are focused on the rates of morbidity and mortality in human populations following an environmental exposure. The linkage between basic biology and disease incidence in environmental health is best described using a tool which is focused on the incidence of disease and which can fully utilize the emerging science [2, 3]. Disease incidence is generally described by counting events (e.g. disease prevalence in a population) or by early, functional failure of an entire organ system (e.g. disease incidence per year). Data endpoints such as these require a different mathematical treatment than the mathematical treatment applied to absorption, distribution and metabolism data endpoints [4]. While the mechanistic basis for understanding environmentally induced disease has progressed rapidly, biologically based mechanistic models of morbidity and mortality lag far behind. This gap in development is partially due to the difficulties in the mathematical treatment of these endpoints and partially due to gaps in scientists' understanding of how the processes occur.

The scientific database for a mechanistic understanding of toxic effects following chemical exposure is probably greatest for the area of carcinogenesis. Several researchers have published work in the area of multistage models for carcinogenesis. The Armitage-Doll model [5] is considered the grandfather of the multistage cancer models. This model has been widely used for the analysis of epidemiological data and

for cancer risk assessment. From the statistical point of view, this model provides a broad class of hazard functions for the analysis of data. Armitage and Doll [6] extended this model to use deterministic birth and death processes on the intermediate state in a two-stage model. Other researchers [7-23] have also contributed significant progress to this field. The models developed use biologically-based information and basic stochastic processes (generally interconnected birth-death processes with immigration and emigration) to reproduce the behavior of cells as they progress through the stages of cancer. Cancer is viewed as a multi-step process in which cells move from a controlled and systematic state of growth into a state of uncontrollable and chaotic growth [24, 25]. The basic assumption – that cancer is a disease of single cells rather than entire organ systems - on which these models were predicated, makes the mathematical modeling of carcinogenesis feasible.

To model a disease process with as much precision as possible, it is imperative to first understand and create a solid foundation for the basic developmental process of a tissue or organ. The linkage is critical in that many of the early markers for carcinogenesis relate back to genes and proteins expressed predominantly during development and cellular replication [26, 27]. Several authors have attempted to create models for the analysis of data from developmental toxicity. Much of this effort has focused on the creation of statistical likelihoods for handling the nested variance observed from developmental toxicology studies or for the longitudinal nature of the data [28-35]. Others have developed statistical models for the probability of a defect focusing on the role of some other type of toxicity (such as maternal toxicity) in the

form of the equations. Leroux et al. [36] developed the first biologically-based, stochastic model of developmental toxicity based on an interconnected birth-death process and using the first and second moments for the number of cells in a second stage of the model as an indicator of the probability of toxicity. The general view of development as modeled by Leroux et al. is a straightforward approach. However, the authors' use of an uncontrolled, birth-death process for cellular growth is both biologically unreasonable (for small numbers of cells) and mathematically uncontrollable allowing for no clear numerical constraints on the size of the resulting organ. This issue will be addressed in a subsequent chapter of the dissertation.

1.3 Delay Differential Equations

The theory of differential equations allows investigators to study various phenomena. But differential equations only take into account the present state of the system. A system where the behavior includes information on former states of the system may force the model closer to reality. These types of systems are commonly called time-delay (time-lag) systems, delay differential equations or functional differential equations. So many natural processes in biology, medicine, chemistry, [37, 38] physics, etc. involve time delays that to ignore them is to ignore reality [39].

Delay differential equations were introduced into mathematical modeling before the 1900s but basics and mathematical formulation did not develop until the 20th century [40]. Although the existence of a delay in a mathematical model may produce a more

realistic model, it is also possible that the delay may induce instability or bad performance into closed-loop schemes [37, 38, 41, 42].

Incorporating time delays into a mathematical model can be a challenge. One issue to consider is the location of the time delay. The critical placement of the delay can yield results that are consistent with the observations or they can lead to very undesirable results. The type of delay incorporated into the system is also very important. Various authors have done extensive work on systems of single delays (see e.g. [43-47]). Another type of delay is a commensurate delay. These are delays where there exists a delays value, t , such that all delays $t_i, (i = 1, \dots, n)$ are rational multiples of t . It is noted that there are some similarities between the commensurate and the single delay case [40]. Multiple delays involve more computations, but the results provide great insight into complicated systems. A mathematical model may incorporate constant time delays or delays that vary with time. The delay can be discrete or continuous. Depending upon the dynamics of the problem to be modeled, a system can be classified as a system of linear functional differential equation or a system of nonlinear functional differential equations.

Once a model has been developed some analyses of the model are usually investigated. These include among other things the stability analysis of the model equation. There are two main subclasses for the stability of functional differential equations: delay-dependent stability and delay-independent stability. Stability criteria that are delay-dependent are a function of at least one of the time lags. On the other hand, delay-independent criteria provide conditions that depend on none of the time

lags from the system under study. For systems that have several delays, investigators have considered the problem of *mixed* delay-independent/delay-dependent stability [40].

1.4 Statement of Problem and Outline

The development of a biologically-based controlled growth and differentiation (CGD) model was inspired by the work of Leroux, Leisenring, Moolgavkar and Fautsman [36]. The original model was developed to address the shortcomings of methods currently used to evaluate the risk of developmental defects in humans as a result of exposure to potential toxic agents. Leroux et al. developed a mathematical model to describe aspects of the dynamic process of organogenesis, based on branching process models of cell kinetics. The work described in this dissertation presents an extension of the model by Leroux et al. [48]. The extended model allows the modeler a greater sense of control in the birth, death and migration of the cellular system at various stages. The CGD model retains the capabilities of the Leroux et al model while adding a higher level of versatility in the model by generalizing the system of ordinary differential equations that describe the developmental process.

Just as the Leroux et al model, the CGD model is biologically- and statistically-based. However, the CGD model incorporates a highly controlled birth and death process for the precursor cells. This formulation limits the number of replications allowed in the development of a tissue or organ and more closely reflects the presence

of a true stem cell population. The biological phenomenon of tissue or organ development is formulated into a mathematical model by making biological assumptions. As stated previously, the Leroux et al. model assumes that there are two basic cell types: uncommitted cells (type- X) and committed cells (type- Y). To allow for mathematical tractability, the Leroux et al. model assumes that cells act independently of one another. The authors also assume that for each cell in the system, only one event (e.g. birth, death, and migration) can take place during a small time interval. The model dictates that cell replications result in daughter cells of the same cell type. Once a cell has become devoted to a particular phenotype, it is assumed that transformation back to the uncommitted stage does not occur. All of the aforementioned assumptions are incorporated into the development of the CGD model except one. While the CGD model does include the two distinct cell types (type- X and type- Y), the model is developed so that the type- X cell population passes through various stages of maturation before differentiation occurs. This modification greatly increases the versatility of the model.

The dissertation is organized as follows. Chapter 2 presents a re-derivation of the Leroux et al. model using the Kolmogorov forward equations. Using this same method, the Leroux et al. model is then extended to formulate the CGD model. A basic comparison between the two models is performed to highlight the most biologically realistic model. Chapter 3 focuses on two applications of the CGD model. In particular, the developmental process of spermatozoon in the male rat reproductive system is investigated. Time delays are added into the CGD system to improve the

level of reality in the model. Then the developmental rates (i.e., birth, death and migration rates) of the CGD model are experimented with to examine the adaptability of the CGD model to hormesis. In Chapter 4, the system of delay differential equations is reformulated and properties of existence and uniqueness are studied. We also include a brief review of the stability theory for linear systems (without delay) using the direct Lyapunov method. This theory is then extended to the case of linear systems with multiple time delays. The stability conditions are expressed in terms of the existence of a positive definite solution to specific Riccati matrix equations. Also in Chapter 4 is an investigation of the stability of a CGD model developed in Chapter 3. Finally, the research presented in this dissertation is summarized in Chapter 5 with a discussion of the results followed by suggested future research directions.

Chapter 2: Model Development

This chapter is a detailed exposition of the development for the Leroux et al. model and the CGD model. Section 2.1 describes the re-derivation of the Leroux et al. model. Using the forward Kolmogorov, this straight forward approach is then extended to derive the CGD model in Section 2.2. The models are compared in Section 2.3 and the advantages of the CGD model are highlighted.

2.1 Leroux et al. Model

In this section, the mathematical model developed by Leroux et al. for the developmental process of a tissue or organ is reviewed. More specifically, it is shown that the same mathematical model, which is a system of ordinary differential equations describing the expectations, squared expectations and expected cross product of cell number, can also be derived using the Kolmogorov forward equations. This modified approach is easily amenable to the expansion of more complicated models of the developmental process including the development of a biologically-based controlled growth and differentiation model for developmental toxicology.

A schematic diagram of the Leroux et al. model is shown in Figure 1.

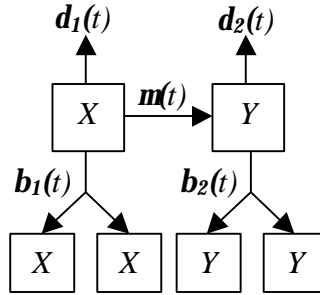


Figure 1: Developmental process of a tissue or organ as described by Leroux et al. The developmental parameters are birth ($b_i(t)$ for $i=1,2$), death ($d_i(t)$ for $i=1,2$) and transformation ($m(t)$).

In this model, the basic developmental process is divided into two main subpopulations. Type- X cells represent cells before commitment to differentiation whereas type- Y cells represent cells that have undergone a transformation and are committed to a phenotype. In addition, the following specific assumptions were made for the mathematical development of the model:

- (i) cells act independently of one another;
- (ii) the probability of more than one event occurring in a single cell in any small time interval Δt is proportional to $o(\Delta t)$;
- (iii) transformation is an irreversible process;
- (iv) a cell in a particular population can only replicate to produce cells of the same population; and
- (v) a malformation results when the number of committed cells ($Y(t)$) is less than a critical number (Y_c) at a specified time, t_c (i.e., $Y(t_c) < Y_c$).

The type- X cell has the option to replicate, die or transform while the type- Y cell may either replicate or die. It is noted that the result of a cell replicating is two daughter cells that are a part of the original population (i.e., the daughter cells are members of the same population as the parent cell). The result of a cell dying is either removal of the cell from the population of properly functioning cells or actual death of a cell. A transformation is the result of a type- X cell moving from the population of uncommitted cells to the population of cells committed to differentiation.

The developmental rates for the system are denoted by $\mathbf{b}_1(t)$ (birth rate in X population), $\mathbf{d}_1(t)$ (death rate in X population), $\mathbf{b}_2(t)$ (birth rate in Y population), $\mathbf{d}_2(t)$ (death rate in Y population) and $\mathbf{m}(t)$ (transformation rate from X population to Y population). The meaning of the rate $\mathbf{b}_1(t)$, for example, is that for the time interval $[t, t + \Delta t)$ where Δt is small, the probability of replication for a type- X cell is $\mathbf{b}_1(t)\Delta t + o(\Delta t)$ where

$$\lim_{\Delta t \rightarrow 0} \frac{o(\Delta t)}{\Delta t} = 0. \quad (2.1)$$

The forward Kolmogorov equation for the transition probability can be expressed generally as

$$P_{\tilde{x}, \tilde{y}, t}(x, y, t + \Delta t) = \Pr[X(t + \Delta t) = x, Y(t + \Delta t) = y \mid X(t) = \tilde{x}, Y(t) = \tilde{y}]. \quad (2.2)$$

Since only certain events, which are summarized in Table 1, can occur in a small time interval Δt , the transition probability $P_{x_0, y_0, 0}$ is given by

$$\begin{aligned}
P_{x_0, y_0, 0}(x, y, t + \Delta t) &= (x-1)\mathbf{b}_1(t)\Delta t P_{x_0, y_0, 0}(x-1, y, t) \\
&\quad + (x+1)\mathbf{d}_1(t)\Delta t P_{x_0, y_0, 0}(x+1, y, t) \\
&\quad + (x+1)\mathbf{m}(t)\Delta t P_{x_0, y_0, 0}(x+1, y-1, t) \\
&\quad + (y-1)\mathbf{b}_2(t)\Delta t P_{x_0, y_0, 0}(x, y-1, t) \\
&\quad + (y+1)\mathbf{d}_2(t)\Delta t P_{x_0, y_0, 0}(x, y+1, t) \\
&\quad + [1 - x\mathbf{b}_1(t)\Delta t - x\mathbf{d}_1(t)\Delta t - x\mathbf{m}(t)\Delta t \\
&\quad \quad - y\mathbf{b}_2(t)\Delta t - y\mathbf{d}_2(t)\Delta t] P_{x_0, y_0, 0}(x, y, t).
\end{aligned} \tag{2.3}$$

Table 1: Single Cell Event Probabilities in Time $[t, t + \Delta t)$ for the Leroux et al. Model

Event	t	$t + \Delta t$	Single cell transition probability
<i>X birth</i>	$x-1, y$	x, y	$\mathbf{b}_1(t) \Delta t P_{x_0, y_0, 0}(x-1, y, t)$
<i>X death</i>	$x+1, y$	x, y	$\mathbf{d}_1(t) \Delta t P_{x_0, y_0, 0}(x+1, y, t)$
<i>Y birth</i>	$x, y-1$	x, y	$\mathbf{b}_2(t) \Delta t P_{x_0, y_0, 0}(x, y-1, t)$
<i>Y death</i>	$x, y+1$	x, y	$\mathbf{d}_2(t) \Delta t P_{x_0, y_0, 0}(x, y+1, t)$
<i>transformation</i>	$x+1, y-1$	x, y	$\mathbf{m}(t) \Delta t P_{x_0, y_0, 0}(x+1, y-1, t)$
<i>no change</i>	x, y	x, y	$\{1 - \mathbf{b}_1(t)\Delta t - \mathbf{d}_1(t)\Delta t - \mathbf{m}(t)\Delta t$ $- \mathbf{b}_2(t)\Delta t - \mathbf{d}_2(t)\Delta t\} P_{x_0, y_0, 0}(x, y, t)$

Recall that for discrete random variables X and Y , the moment generating function for a time interval of length Δt given the initial values x_0 and y_0 is given by

$$E[X^n(t + \Delta t)Y^p(t + \Delta t) | X(0) = x_0, Y(0) = y_0] = \sum_x \sum_y x^n y^p P_{x_0, y_0, 0}(x, y, t + \Delta t). \tag{2.4}$$

Hence, using equations (2.3) and (2.4) the first moment (mean) for X is defined as

$$\begin{aligned}
&E[X^1(t + \Delta t)Y^0(t + \Delta t) | X(0) = x_0, Y(0) = y_0] \\
&= E[X(t + \Delta t) | X(0) = x_0, Y(0) = y_0]
\end{aligned} \tag{2.5}$$

$$= \sum_x \sum_y x P_{x_0, y_0, 0}(x, y, t + \Delta t) \quad (2.6)$$

$$\begin{aligned}
&= \sum_x \sum_y [x(x-1)\mathbf{b}_1(t)\Delta t P_{x_0, y_0, 0}(x-1, y, t) + x(x+1)\mathbf{d}_1(t)\Delta t P_{x_0, y_0, 0}(x+1, y, t) \\
&\quad + x(x+1)\mathbf{m}(t)\Delta t P_{x_0, y_0, 0}(x+1, y-1, t) \\
&\quad + x(y-1)\mathbf{b}_2(t)\Delta t P_{x_0, y_0, 0}(x, y-1, t) + x(y+1)\mathbf{d}_2(t)\Delta t P_{x_0, y_0, 0}(x, y+1, t) \\
&\quad + x[1-x\mathbf{b}_1(t)\Delta t - x\mathbf{d}_1(t)\Delta t - x\mathbf{m}(t)\Delta t - y\mathbf{b}_2(t)\Delta t - y\mathbf{d}_2(t)\Delta t] P_{x_0, y_0, 0}(x, y, t).
\end{aligned} \quad (2.7)$$

The first term in equation (2.7) can be simplified by rescaling the summation indices as follows:

$$\sum_x \sum_y x(x-1)\mathbf{b}_1(t)\Delta t P_{x_0, y_0, 0}(x-1, y, t) = \sum_{x'} \sum_y (x'+1)x'\mathbf{b}_1(t)\Delta t P_{x_0, y_0, 0}(x', y, t) \quad (2.8)$$

$$= \sum_{x'} \sum_y x'^2 \mathbf{b}_1(t)\Delta t P_{x_0, y_0, 0}(x', y, t) + \sum_{x'} \sum_y x' \mathbf{b}_1(t)\Delta t P_{x_0, y_0, 0}(x', y, t) \quad (2.9)$$

$$= E[X^2(t)]\mathbf{b}_1(t)\Delta t + E[X(t)]\mathbf{b}_1(t)\Delta t, \quad (2.10)$$

where $E[X^2(t)]$ is used to denote $E[X^2(t) | X(0) = x_0, Y(0) = y_0]$. Proceeding in a similar manner for the remaining terms in equation (2.7) leads to

$$E[X(t + \Delta t)] = \{\mathbf{b}_1(t)\Delta t - \mathbf{d}_1(t)\Delta t - \mathbf{m}(t)\Delta t + 1\} E[X(t)]. \quad (2.11)$$

Subtracting $E[X(t)]$ from both sides of equation (2.11) and then dividing both sides by Δt yields

$$\frac{E[X(t + \Delta t)] - E[X(t)]}{\Delta t} = \{\mathbf{b}_1(t) - \mathbf{d}_1(t) - \mathbf{m}(t)\} E[X(t)], \quad (2.12)$$

which, in the limit as Δt goes to zero, gives

$$\frac{d}{dt} E[X(t)] = \{\mathbf{b}_1(t) - \mathbf{d}_1(t) - \mathbf{m}(t)\} E[X(t)]. \quad (2.13)$$

The expected value (mean) for the random variable Y can be derived in a similar manner. The variances and the covariance (i.e., $E[X^2(t) | X(0) = x_0, Y(0) = y_0]$, $E[Y^2(t) | X(0) = x_0, Y(0) = y_0]$ and $E[X(t)Y(t) | X(0) = x_0, Y(0) = y_0]$, respectively) are also calculated by following the same procedure. Therefore, the system of ordinary differential equations describing the means, variances and covariance for the number of cells in states X and Y at any time t is given by

$$\frac{d}{dt} E[X(t)] = \{\mathbf{b}_1(t) - \mathbf{d}_1(t) - \mathbf{m}(t)\} E[X(t)] \quad (2.14)$$

$$\frac{d}{dt} E[Y(t)] = \{\mathbf{b}_2(t) - \mathbf{d}_2(t)\} E[Y(t)] + \mathbf{m}(t) E[X(t)] \quad (2.15)$$

$$\begin{aligned} \frac{d}{dt} E[X^2(t)] &= 2\{\mathbf{b}_1(t) - \mathbf{d}_1(t) - \mathbf{m}(t)\} E[X^2(t)] \\ &\quad + \{\mathbf{b}_1(t) + \mathbf{d}_1(t) + \mathbf{m}(t)\} E[X(t)] \end{aligned} \quad (2.16)$$

$$\begin{aligned} \frac{d}{dt} E[Y^2(t)] &= 2\{\mathbf{b}_2(t) - \mathbf{d}_2(t)\} E[Y^2(t)] + \{\mathbf{b}_2(t) + \mathbf{d}_2(t)\} E[Y(t)] \\ &\quad + \mathbf{m}(t) E[X(t)] + 2\mathbf{m}(t) E[X(t)Y(t)] \end{aligned} \quad (2.17)$$

$$\begin{aligned} \frac{d}{dt} E[X(t)Y(t)] &= \{\mathbf{b}_1(t) - \mathbf{d}_1(t) + \mathbf{b}_2(t) - \mathbf{d}_2(t) - \mathbf{m}(t)\} E[X(t)Y(t)] \\ &\quad - \mathbf{m}(t) E[X(t)] + \mathbf{m}(t) E[X^2(t)]. \end{aligned} \quad (2.18)$$

The above mathematical model is identical to the one derived by Leroux et al. in which the authors considered a partial differential equation for the generating function for $X(t)$ and $Y(t)$. Particularly, Leroux et al. chose the generating function for $X(t)$ and $Y(t)$ to be

$$\mathbf{f}(u, v, t; x_0, y_0, t_0) = \sum_x \sum_y u^x v^y P(x, y, t; x_0, y_0, t_0). \quad (2.19)$$

Using the Kolmogorov forward equations, Leroux et al. derived the following partial differential equation for the generating function:

$$\begin{aligned} \frac{\partial}{\partial t} \mathbf{j}(u, v, t) = & u \frac{\partial}{\partial u} \mathbf{j}(u, v, t) [(u-1)\mathbf{I}_1(t) \\ & + (u^{-1}-1)\mathbf{m}_1(t) + (u^{-1}v-1)v(t)] \\ & + v \frac{\partial}{\partial v} \mathbf{j}(u, v, t) [(v-1)\mathbf{I}_2(t) + (v^{-1}-1)\mathbf{m}_2(t)]. \end{aligned} \quad (2.20)$$

In the case of constant rates, the system of differential equations (2.14)-(2.18) is a linear system of ordinary differential equations with a constant coefficient matrix of the form

$$\bar{\mathbf{y}}'(t) = A\bar{\mathbf{y}}(t) \quad (2.21)$$

where $\bar{\mathbf{y}}(t) = (E[X(t)], E[Y(t)], E[X^2(t)], E[Y^2(t)], E[X(t)Y(t)])^T$ and

$$A = \begin{bmatrix} \mathbf{b}_1 - \mathbf{d}_1 - \mathbf{m} & 0 & 0 & 0 & 0 \\ \mathbf{m} & \mathbf{b}_2 - \mathbf{d}_2 & 0 & 0 & 0 \\ \mathbf{b}_1 + \mathbf{d}_1 + \mathbf{m} & 0 & 2(\mathbf{b}_1 - \mathbf{d}_1 - \mathbf{m}) & 0 & 0 \\ \mathbf{m} & \mathbf{b}_2 + \mathbf{d}_2 & 0 & 2(\mathbf{b}_2 - \mathbf{d}_2) & 2\mathbf{m} \\ -\mathbf{m} & 0 & \mathbf{m} & 0 & \mathbf{b}_1 - \mathbf{d}_1 - \mathbf{m} + \mathbf{b}_2 - \mathbf{d}_2 \end{bmatrix}. \quad (2.22)$$

The exact solution can be written in terms of the eigenvalues and eigenvectors of the matrix A (see e.g., [49]).

2.2 Controlled Growth and Differentiation Model

As already discussed elsewhere in this dissertation, the mathematical model formulated by Leroux et al. is both biologically and mathematically restrictive because it allows no constraint on the size of the resulting organ or tissue and it is difficult to

expand their model to larger, more detailed biological systems with intermediate stages. To this end, attention is focused on modifications of the Leroux et al. model that put greater emphasis on the control of growth and differentiation. The CGD model assumes that daughter cells advance to the next stage of development while the Leroux et al. model assumes that the daughter cells rejoin the original population of the parent cells. This assumption of a linear birth-death process in both the type- X and type- Y of the Leroux et al. model is not adopted in the CGD model. More specifically, a multistate developmental process with separate growth phases in the type- X cells and a linear birth-death process on the type- Y cells are considered. A schematic diagram of the CGD model is illustrated in Figure 2.

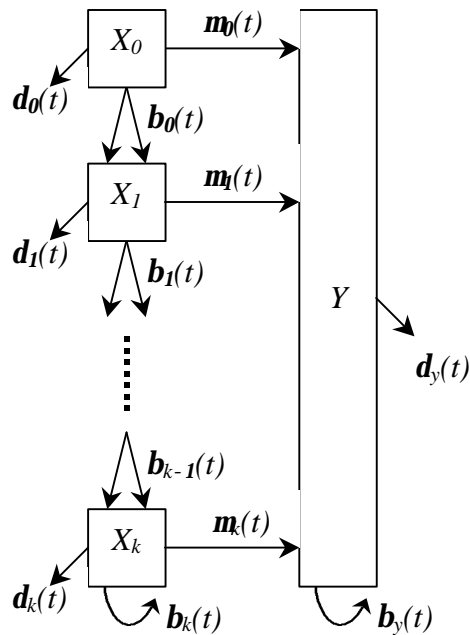


Figure 2: A general CGD system with $k+2$ states. This system allows the cells to go through various levels of maturation before committing to the differentiation process.

In this system, there are $k+1$ specific type- X populations denoted as type- X_i , where $i = 0, 1, 2, \dots, k$. Each of the type- X cell populations represents cells prior to commitment to differentiation. Moreover, as a cell moves from one stage, for example from X_i , to the next stage, X_{i+1} , it matures in the developmental process. By setting $\mathbf{m}_i = 0$, for one or more values of i , greater control is allowed in the type- X cells prior to differentiation. Eventually, the uncommitted cell will undergo a change and join the population of cells already committed to performing a specific function of a tissue or organ. This process by which an uncommitted cell becomes a cell committed to differentiation is termed *transformation*. Type- Y cells denote the population of cells committed to differentiation. If the first moments in the CGD model are represented by n random variables, then there are n first moments, n second (squared) moments, and $\frac{n(n-1)}{2}$ second (cross product) moments. The following equation can be used to determine the dimension of the CGD model when the number of random variables is specified

$$E_{CGD} = 2n + \frac{n(n-1)}{2}. \quad (2.23)$$

The assumptions and the parameters in the model have the same basic definition as those in the Leroux et al. model (see Section 2.1). More specifically, for each type- X_i cell, one of three outcomes is possible: division of the cell into two type- X_{i+1} daughter cells (i.e., a birth in the type- X_i population), transformation of the cell into the type- Y population or death of the cell. Cells of the type- Y population are allowed only

one of two outcomes: division into two type- Y daughter cells or death prior to division. In the type- Y population death is understood to include actual death of a cell or removal from the pool of fully committed cells contributing to the development and function of the tissue or organ in consideration.

The developmental rates are again denoted by $\mathbf{b}_i(t)$ (birth rate in a X_i population), $\mathbf{d}_i(t)$ (death rate in a X_i population), $\mathbf{b}_y(t)$ (birth rate in the Y population), $\mathbf{d}_y(t)$ (death rate in the Y population) and $\mathbf{m}_i(t)$ (transformation rate from the X_i population to the Y population) where $i = 0, 1, \dots, k$. Just as before, it is assumed that the probability of more than one event occurring during small time interval Δt is $o(\Delta t)$, which by condition (2.1), is negligible. Table 2 includes all of the possible events for a single cell in the CGD developmental system.

Table 2: Single Cell Event Probabilities in Time $[t, t + \Delta t)$ for the CGD Model

<i>Event</i>	<i>t</i>	<i>t+Dt</i>	<i>Single cell transition probability</i>
<i>birth from</i> X_i to X_{i+1} ($i=0, \dots, k-1$)	$x_0, \dots, x_i+1, x_{i+1}-2, \dots, y$	x_0, \dots, x_k, y	$\mathbf{b}_i(t) \Delta t P(x_0, \dots, x_i+1, x_{i+1}-2, \dots, y, t)$
<i>birth in state</i> X_k	x_0, \dots, x_k-1, y	x_0, \dots, x_k, y	$\mathbf{b}_k(t) \Delta t P(x_0, \dots, x_k-1, y, t)$
<i>death in</i> X_i ($i=0, \dots, k$)	$x_0, \dots, x_i+1, \dots, x_k, y$	x_0, \dots, x_k, y	$\mathbf{d}_i(t) \Delta t P(x_0, \dots, x_i+1, \dots, x_k, y, t)$
<i>transformation</i> <i>from</i> X_i <i>to</i> Y ($i=0, \dots, k$)	$x_0, \dots, x_i+1, \dots, x_k, y-1$	x_0, \dots, x_k, y	$\mathbf{m}_i(t) \Delta t P(x_0, \dots, x_i+1, \dots, x_k, y-1, t)$
<i>birth in state</i> Y	$x_0, \dots, x_k, y-1$	x_0, \dots, x_k, y	$\mathbf{b}_y(t) \Delta t P(x_0, \dots, x_k, y-1, t)$
<i>death in state</i> Y	$x_0, \dots, x_k, y+1$	x_0, \dots, x_k, y	$\mathbf{d}_y(t) \Delta t P(x_0, \dots, x_k, y+1, t)$
<i>no change</i>	x_0, \dots, x_k, y	x_0, \dots, x_k, y	$\left\{ 1 - \left[\sum_{i=0}^k (\mathbf{b}_i + \mathbf{d}_i + \mathbf{m}_i) + \mathbf{b}_y + \mathbf{d}_y \right] \Delta t \right\}$ $\cdot P(x_0, \dots, x_k, y, t)$

Based on the information presented in Table 2, only certain events may occur in a small time interval, Δt . Thus, the transition probability function for a CGD developmental system is given by

$$\begin{aligned}
& P_{x_{00}, \dots, x_{k0}, y_0, 0}(x_0, \dots, x_k, y, t + \Delta t) \\
&= \sum_{i=0}^{k-1} (x_i + 1) \mathbf{b}_i(t) \Delta t P_{x_{00}, \dots, x_{k0}, y_0, 0}(x_0, \dots, x_i + 1, x_{i+1} - 2, \dots, x_k, y, t) \\
&+ (x_k - 1) \mathbf{b}_k(t) \Delta t P_{x_{00}, \dots, x_{k0}, y_0, 0}(x_0, \dots, x_k - 1, y, t) \\
&+ \sum_{i=0}^k (x_i + 1) \mathbf{d}_i(t) \Delta t P_{x_{00}, \dots, x_{k0}, y_0, 0}(x_0, \dots, x_i + 1, \dots, x_k, y, t) \\
&+ \sum_{i=0}^k (x_i + 1) \mathbf{m}_i(t) \Delta t P_{x_{00}, \dots, x_{k0}, y_0, 0}(x_0, \dots, x_i + 1, \dots, x_k, y - 1, t) \\
&+ (y - 1) \mathbf{b}_y(t) \Delta t P_{x_{00}, \dots, x_{k0}, y_0, 0}(x_0, \dots, x_k, y - 1, t) \\
&+ (y + 1) \mathbf{d}_y(t) \Delta t P_{x_{00}, \dots, x_{k0}, y_0, 0}(x_0, \dots, x_k, y + 1, t) \\
&+ \left[1 - \sum_{i=0}^k x_i (\mathbf{b}_i(t) + \mathbf{d}_i(t) + \mathbf{m}_i(t)) \Delta t - y (\mathbf{b}_y(t) + \mathbf{d}_y(t)) \Delta t \right] P_{x_{00}, \dots, x_{k0}, y_0, 0}(x_0, \dots, x_k, y, t).
\end{aligned} \tag{2.24}$$

Since an objective of the CGD model is to estimate the average number of cells that accumulate in stage Y at a particular time of the developmental process, the expected value (first moment) for each random variable is calculated. This is done in the same manner as for the re-derivation the Leroux et al. model in Section 2.1.

For an arbitrary discrete random variable X_j and a discrete random variable Y , the moments for a time interval of length Δt given the initial values $x_{00}, x_{10}, \dots, x_{k-1,0}, x_{k0}$ and y_0 is defined to be

$$\begin{aligned}
& E \left[X_j^n(t + \Delta t) Y^p(t + \Delta t) \mid X_j(0) = x_{j0}, Y(0) = y_0 \right] \\
&= \sum_{x_0} \sum_{x_1} \dots \sum_{x_k} \sum_y x_j^n y^p P_{x_{00}, \dots, x_{k0}, y_0, 0}(x_0, x_1, \dots, x_k, y, t + \Delta t).
\end{aligned} \tag{2.25}$$

Following the same process as presented in Section 2.1, the first moment for the random variable $X_j, j = 0, 1, \dots, k - 1$, of the CGD model is given by

$$\frac{d}{dt}E[X_j(t)] = -\{\mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t)\}E[X_j(t)] + 2\mathbf{b}_{j-1}(t)E[X_{j-1}(t)]. \quad (2.26)$$

Detailed calculations of the expected value of X_j , for $j=0,1,\dots,k-1$ is provided below. The following process can be repeated to obtain the remaining first and second moments. Based on equation (2.4) the expected value for the random variable X_j can be written as

$$\begin{aligned} & E[X_j(t + \Delta t)] \\ &= \sum_{x_0} \sum_{x_1} \dots \sum_{x_k} \sum_y \left\{ x_j \sum_{\substack{i=0 \\ i \neq j-1 \\ i \neq j}}^{k-1} (x_i + 1) \mathbf{b}_i(t) \Delta t P(x_0, \dots, x_i + 1, x_{i+1} - 2, \dots, x_k, y, t) \right. \\ &+ x_j (x_{j-1} + 1) \mathbf{b}_{j-1}(t) \Delta t P(x_0, \dots, x_{j-1} + 1, x_j - 2, \dots, x_k, y, t) \\ &+ x_j (x_j + 1) \mathbf{b}_j(t) \Delta t P(x_0, \dots, x_j + 1, x_{j+1} - 2, \dots, x_k, y, t) \\ &+ x_j (x_k - 1) \mathbf{b}_k(t) \Delta t P(x_0, \dots, x_k - 1, y, t) \\ &+ x_j \sum_{\substack{i=0 \\ i \neq j-1 \\ i \neq j}}^k (x_i + 1) \mathbf{d}_i(t) \Delta t P(x_0, \dots, x_i + 1, \dots, x_k, y, t) \\ &+ x_j (x_{j-1} + 1) \mathbf{d}_{j-1}(t) \Delta t P(x_0, \dots, x_{j-1} + 1, \dots, x_k, y, t) \\ &+ x_j (x_j + 1) \mathbf{d}_j(t) \Delta t P(x_0, \dots, x_j + 1, \dots, x_k, y, t) \\ &+ x_j \sum_{\substack{i=0 \\ i \neq j-1 \\ i \neq j}}^k (x_i + 1) \mathbf{m}_i(t) \Delta t P(x_0, \dots, x_i + 1, \dots, x_k, y - 1, t) \\ &+ x_j (x_{j-1} + 1) \mathbf{m}_{j-1}(t) \Delta t P(x_0, \dots, x_{j-1} + 1, \dots, x_k, y - 1, t) \\ &+ x_j (x_j + 1) \mathbf{m}_j(t) \Delta t P(x_0, \dots, x_j + 1, \dots, x_k, y - 1, t) \\ &+ x_j (y - 1) \mathbf{b}_y(t) \Delta t P(x_0, \dots, x_k, y - 1, t) + x_j (y + 1) \mathbf{d}_y \Delta t P(x_0, \dots, x_k, y + 1, t) \quad (2.27) \\ &+ x_j \left[1 - \sum_{i=0}^k x_i (\mathbf{b}_i(t) + \mathbf{d}_i(t) + \mathbf{m}_i(t)) \Delta t - y (\mathbf{b}_y(t) + \mathbf{d}_y(t)) \Delta t \right] P(x_0, \dots, x_k, y, t) \left. \right\}. \end{aligned}$$

At this point all the probability density functions are rescaled. Also denote $P(x_0, \dots, x_k, y, t)$ simply by

$$P(\bar{x}, y, t). \quad (2.28)$$

Thus, equation (2.27) becomes

$$\begin{aligned}
E[X_j(t + \Delta t)] &= \sum_{\bar{x}} \sum_y \left\{ x_j \sum_{\substack{i=0 \\ i \neq j-1 \\ i \neq j}}^{k-1} x_i \mathbf{b}_i(t) \Delta t P(\bar{x}, y, t) \right. \\
&\quad + (x_j + 2)x_{j-1} \mathbf{b}_{j-1}(t) \Delta t P(\bar{x}, y, t) \\
&\quad + (x_j - 1)x_j \mathbf{b}_j(t) \Delta t P(\bar{x}, y, t) + x_j x_k \mathbf{b}_k(t) \Delta t P(\bar{x}, y, t) \\
&\quad + x_j \sum_{\substack{i=0 \\ i \neq j-1 \\ i \neq j}}^k x_i \mathbf{d}_i(t) \Delta t P(\bar{x}, y, t) + x_j x_{j-1} \mathbf{d}_{j-1}(t) \Delta t P(\bar{x}, y, t) \\
&\quad + (x_j - 1)x_j \mathbf{d}_j(t) \Delta t P(\bar{x}, y, t) \\
&\quad + x_j \sum_{\substack{i=0 \\ i \neq j-1 \\ i \neq j}}^k x_i \mathbf{m}_i(t) \Delta t P(\bar{x}, y, t) + x_j x_{j-1} \mathbf{m}_{j-1}(t) \Delta t P(\bar{x}, y, t) \\
&\quad + (x_j - 1)x_j \mathbf{m}_j(t) \Delta t P(\bar{x}, y, t) \\
&\quad + x_j y \mathbf{b}_y(t) \Delta t P(\bar{x}, y, t) + x_j y \mathbf{d}_y(t) \Delta t P(\bar{x}, y, t) \\
&\quad + x_j P(\bar{x}, y, t) - x_j \sum_{i=0}^k x_i (\mathbf{b}_i(t) + \mathbf{d}_i(t) + \mathbf{m}_i(t)) \Delta t P(\bar{x}, y, t) \\
&\quad \left. - x_j y (\mathbf{b}_y(t) + \mathbf{d}_y(t)) \Delta t P(\bar{x}, y, t) \right\}. \quad (2.29)
\end{aligned}$$

Further simplifications reduce to

$$\begin{aligned}
E[X_j(t + \Delta t)] &= \sum_{\bar{x}} \sum_y \left\{ 2\mathbf{b}_{j-1}(t) \Delta t x_{j-1} P(\bar{x}, y, t) \right. \\
&\quad \left. - (\mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t)) \Delta t x_j P(\bar{x}, y, t) + x_j P(\bar{x}, y, t) \right\}. \quad (2.30)
\end{aligned}$$

Recall that the mathematical definition of the moments and correlation's of the discrete random variables X_j and Y at time $t + \Delta t$ is given by

$$\begin{aligned}
& E\left[X_j^n(t+\Delta t)Y^p(t+\Delta t) \mid X_j(0) = x_{j0}, Y(0) = y_0\right] \\
&= \sum_{x_0} \sum_{x_1} \dots \sum_{x_k} \sum_y x_j^n y^p P_{x_{00}, x_{10}, \dots, x_{k-1,0}, x_{k0}, y_0, 0}(x_0, x_1, \dots, x_k, y, t + \Delta t). \quad (2.31)
\end{aligned}$$

Thus with $n = 1$ and $p = 0$, equation (2.30) reduces to

$$\begin{aligned}
E[X_j(t+\Delta t)] &= 2\mathbf{b}_{j-1}(t)\Delta t E[X_{j-1}(t)] \\
&\quad -(\mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t))\Delta t E[X_j(t)] + E[X_j(t)]. \quad (2.32)
\end{aligned}$$

Finally, subtracting $E[X_j(t)]$ from both sides of equation (2.32), dividing by Δt and

letting Δt approaches zero yields

$$\begin{aligned}
& \lim_{\Delta t \rightarrow 0} \left[\frac{E[X_j(t+\Delta t)] - E[X_j(t)]}{\Delta t} \right] \\
&= 2\mathbf{b}_{j-1}(t)E[X_{j-1}(t)] - (\mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t))E[X_j(t)], \quad (2.33)
\end{aligned}$$

or equivalently

$$\frac{d}{dt} E[X_j(t)] = 2\mathbf{b}_{j-1}(t)E[X_{j-1}(t)] - (\mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t))E[X_j(t)]. \quad (2.34)$$

The remaining first moments and the second moments for the discrete random variables are calculated in a similar manner. The result is the following system of ordinary differential equations with time dependent developmental rates (unless otherwise stated, $j = 0, 1, 2, \dots, k-1$):

$$\frac{d}{dt} E[X_j(t)] = -\{\mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t)\}E[X_j(t)] + 2\mathbf{b}_{j-1}(t)E[X_{j-1}(t)] \quad (2.35)$$

$$\frac{d}{dt} E[X_k(t)] = \{\mathbf{b}_k(t) - \mathbf{d}_k(t) - \mathbf{m}_k(t)\}E[X_k(t)] + 2\mathbf{b}_{k-1}(t)E[X_{k-1}(t)] \quad (2.36)$$

$$\frac{d}{dt} E[Y(t)] = \{\mathbf{b}_y(t) - \mathbf{d}_y(t)\}E[Y(t)] + \sum_{i=0}^k \mathbf{m}_i(t)E[X_i(t)] \quad (2.37)$$

$$\begin{aligned}
\frac{d}{dt}E[X_j^2(t)] &= -2\{\mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t)\}E[X_j^2(t)] \\
&\quad + \{\mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t)\}E[X_j(t)] \\
&\quad + 4\mathbf{b}_{j-1}(t)E[X_{j-1}(t)] + 4\mathbf{b}_{j-1}(t)E[X_{j-1}(t)X_j(t)]
\end{aligned} \tag{2.38}$$

$$\begin{aligned}
\frac{d}{dt}E[X_k^2(t)] &= 2\{\mathbf{b}_k(t) - \mathbf{d}_k(t) - \mathbf{m}_k(t)\}E[X_k^2(t)] \\
&\quad + \{\mathbf{b}_k(t) + \mathbf{d}_k(t) + \mathbf{m}_k(t)\}E[X_k(t)] \\
&\quad + 4\mathbf{b}_{k-1}(t)E[X_{k-1}(t)] + 4\mathbf{b}_{k-1}(t)E[X_{k-1}(t)X_k(t)]
\end{aligned} \tag{2.39}$$

$$\begin{aligned}
\frac{d}{dt}E[Y^2(t)] &= 2\{\mathbf{b}_y(t) - \mathbf{d}_y(t)\}E[Y^2(t)] \\
&\quad + \{\mathbf{b}_y(t) + \mathbf{d}_y(t)\}E[Y(t)] \\
&\quad + \sum_{i=0}^k \mathbf{m}_i E[X_i(t)] + 2\sum_{i=0}^k \mathbf{m}_i E[X_i(t)Y(t)]
\end{aligned} \tag{2.40}$$

$$\begin{aligned}
\frac{d}{dt}E[X_l(t)X_j(t)] &= -\{\mathbf{b}_l(t) + \mathbf{d}_l(t) + \mathbf{m}_l(t) + \mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t)\}E[X_l(t)X_j(t)] \\
&\quad + 2\mathbf{b}_{l-1}(t)E[X_{l-1}(t)X_j(t)] + 2\mathbf{b}_{j-1}(t)E[X_l(t)X_{j-1}(t)]
\end{aligned} \tag{2.41}$$

where $l, j = 0, 1, 2, \dots, k$ and l, j are not consecutive integers $l \neq j$

$$\begin{aligned}
\frac{d}{dt}E[X_{j-1}(t)X_j(t)] &= -2\mathbf{b}_{j-1}(t)E[X_{j-1}(t)] + 2\mathbf{b}_{j-1}(t)E[X_{j-1}^2(t)] \\
&\quad + 2\mathbf{b}_{j-2}(t)E[X_{j-2}(t)X_j(t)] \\
&\quad - \{\mathbf{b}_{j-1}(t) + \mathbf{d}_{j-1}(t) + \mathbf{m}_{j-1}(t) + \mathbf{b}_j(t) + \mathbf{d}_j(t) + \mathbf{m}_j(t)\}E[X_{j-1}(t)X_j(t)]
\end{aligned} \tag{2.42}$$

$$\begin{aligned}
\frac{d}{dt}E[X_{k-1}(t)X_k(t)] &= -2\mathbf{b}_{k-1}(t)E[X_{k-1}(t)] + 2\mathbf{b}_{k-1}(t)E[X_{k-1}^2(t)] \\
&\quad + 2\mathbf{b}_{k-2}(t)E[X_{k-2}(t)X_k(t)] \\
&\quad + \{\mathbf{b}_k(t) - \mathbf{d}_k(t) - \mathbf{m}_k(t) - \mathbf{b}_{k-1}(t) - \mathbf{d}_{k-1}(t) - \mathbf{m}_{k-1}(t)\}E[X_{k-1}(t)X_k(t)]
\end{aligned} \tag{2.43}$$

$$\begin{aligned}
\frac{d}{dt}E[X_j(t)Y(t)] &= \{ \mathbf{b}_y(t) - \mathbf{d}_y(t) - \mathbf{b}_j(t) - \mathbf{d}_j(t) - \mathbf{m}_j(t) \} E[X_j(t)Y(t)] \\
&+ 2\mathbf{b}_{j-1}(t)E[X_{j-1}(t)Y(t)] - \mathbf{m}_j(t)E[X_j(t)] \\
&+ \sum_{i=0}^k \mathbf{m}_i(t)E[X_j(t)X_i(t)]. \quad \text{for } j = 0, 1, 2, \dots, k-1
\end{aligned} \tag{2.44}$$

$$\begin{aligned}
\frac{d}{dt}E[X_k(t)Y(t)] &= \{ \mathbf{b}_y(t) - \mathbf{d}_y(t) + \mathbf{b}_k(t) - \mathbf{d}_k(t) - \mathbf{m}_k(t) \} E[X_k(t)Y(t)] \\
&+ 2\mathbf{b}_{k-1}(t)E[X_{k-1}(t)Y(t)] - \mathbf{m}_k(t)E[X_k(t)] \\
&+ \sum_{i=0}^k \mathbf{m}_i(t)E[X_k(t)X_i(t)].
\end{aligned} \tag{2.45}$$

2.3 Comparison of Models

In this section, the Leroux et al. model and the CGD model are examined and compared in an effort to highlight the most useful and biologically realistic model for the developmental process as it relates to animals. For a description on the experimental aspects and how the *in vitro* data were used to estimate cell kinetic rates in both models see the Leroux et al. paper. In addition, all computations were done using computer codes written in the MATLAB/Simulink environment (The Math Works, Inc., Natick, Massachusetts).

The two models are not only biologically and mathematically different but the model proposed by Leroux et al. is not a submodel of the CGD model since a linear birth process is not assumed. However, using specific birth, death or transformation rates in the CGD model creates a model that can give similar predictions as those given

by the Leroux et al. model. Specifically, consider the very simple developmental CGD model depicted in Figure 3.

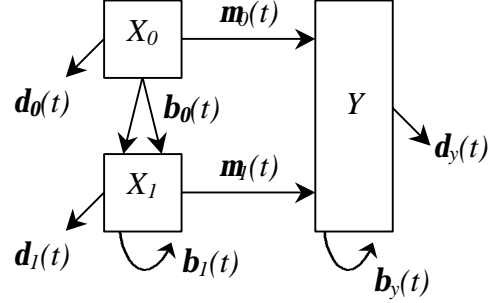


Figure 3: Basic CGD model that predicts similar expectations and variances as the Leroux et al. model at time $t=5$. Table 3 lists all of the developmental rates associated with this particular CGD model.

The system of ordinary differential equations describing the means, variances and covariances for the number of cells in states X_j ($j = 0,1$) and Y is given below.

$$\frac{d}{dt} E[X_0(t)] = -\{\mathbf{b}_0(t) + \mathbf{d}_0(t) + \mathbf{m}_0(t)\} E[X_0(t)] \quad (2.46)$$

$$\frac{d}{dt} E[X_1(t)] = \{\mathbf{b}_1(t) - \mathbf{d}_1(t) - \mathbf{m}_1(t)\} E[X_1(t)] + 2\mathbf{b}_0(t) E[X_0(t)] \quad (2.47)$$

$$\frac{d}{dt} E[Y(t)] = \{\mathbf{b}_y(t) - \mathbf{d}_y(t)\} E[Y(t)] + \mathbf{m}_0(t) E[X_0(t)] + \mathbf{m}_1(t) E[X_1(t)] \quad (2.48)$$

$$\begin{aligned} \frac{d}{dt} E[X_0^2(t)] = & -2\{\mathbf{b}_0(t) + \mathbf{d}_0(t) + \mathbf{m}_0(t)\} E[X_0^2(t)] \\ & + \{\mathbf{b}_0(t) + \mathbf{d}_0(t) + \mathbf{m}_0(t)\} E[X_0(t)] \end{aligned} \quad (2.49)$$

$$\begin{aligned}
\frac{d}{dt}E[X_1^2(t)] &= 2\{\mathbf{b}_1(t) - \mathbf{d}_1(t) - \mathbf{m}_1(t)\}E[X_1^2(t)] \\
&\quad + \{\mathbf{b}_1(t) + \mathbf{d}_1(t) + \mathbf{m}_1(t)\}E[X_1(t)] \\
&\quad + 4\mathbf{b}_0(t)E[X_0(t)] + 4\mathbf{b}_0(t)E[X_0(t)X_1(t)]
\end{aligned} \tag{2.50}$$

$$\begin{aligned}
\frac{d}{dt}E[Y^2(t)] &= 2\{\mathbf{b}_y(t) - \mathbf{d}_y(t)\}E[Y^2(t)] + \{\mathbf{b}_y(t) + \mathbf{d}_y(t)\}E[Y(t)] \\
&\quad + \mathbf{m}_0E[X_0(t)] + \mathbf{m}_1E[X_1(t)] \\
&\quad + 2\{\mathbf{m}_0E[X_0(t)Y(t)] + \mathbf{m}_1E[X_1(t)Y(t)]\}
\end{aligned} \tag{2.51}$$

$$\begin{aligned}
\frac{d}{dt}E[X_0(t)X_1(t)] &= -2\mathbf{b}_0(t)E[X_0(t)] + 2\mathbf{b}_0(t)E[X_0^2(t)] \\
&\quad + \{\mathbf{b}_1(t) - \mathbf{d}_1(t) - \mathbf{m}_1(t) - \mathbf{b}_0(t) - \mathbf{d}_0(t) - \mathbf{m}_0(t)\}E[X_0(t)X_1(t)]
\end{aligned} \tag{2.52}$$

$$\begin{aligned}
\frac{d}{dt}E[X_0(t)Y(t)] &= \{\mathbf{b}_y(t) - \mathbf{d}_y(t) - \mathbf{b}_0(t) - \mathbf{d}_0(t) - \mathbf{m}_0(t)\}E[X_0(t)Y(t)] \\
&\quad - \mathbf{m}_0(t)E[X_0(t)] + \mathbf{m}_0(t)E[X_0^2(t)] + \mathbf{m}_1(t)E[X_0(t)X_1(t)]
\end{aligned} \tag{2.53}$$

$$\begin{aligned}
\frac{d}{dt}E[X_1(t)Y(t)] &= \{\mathbf{b}_y(t) - \mathbf{d}_y(t) + \mathbf{b}_1(t) - \mathbf{d}_1(t) - \mathbf{m}_1(t)\}E[X_1(t)Y(t)] \\
&\quad - \mathbf{m}_1(t)E[X_1(t)] + \mathbf{m}_1(t)E[X_1^2(t)] \\
&\quad + \mathbf{m}_0(t)E[X_0(t)X_1(t)] + 2\mathbf{b}_0(t)E[X_0(t)Y(t)]
\end{aligned} \tag{2.54}$$

At time $t = 0$, the variance and covariance of each random variable is set equal to zero. Thus, using the parameters given in Table 3, the CGD and the Leroux et al. model predict similar expectations and variances at time $t = 5$.

Table 3: Parameters Used to Predict Similar Results Among the Two Models

	Leroux et al. Model		CGD Model		
	X	Y	X_0	X_I	Y
Initial number of cells (time $t=0$)	1.68×10^5	0	1.68×10^5	0	0
Birth rate, \mathbf{b}	0.4	0.4	0.4	0.4	0.4
Death rate, \mathbf{d}	0.01	0.01	0.01	0.01	0.01
Transformation rate, \mathbf{m}	0.15	--	0.15	0.15	--
Expected number of cells (time $t=5$)	5.578×10^5	6.23×10^5	1.022×10^4	5.476×10^5	6.23×10^5
Total number of cells	1.181×10^6		1.181×10^6		

However, one major difference between the two models is the ability of the CGD model to deplete the population of X cells under constant rates while still maintaining the same distribution of Y cells. This can be illustrated using the simple CGD model in Figure 4.

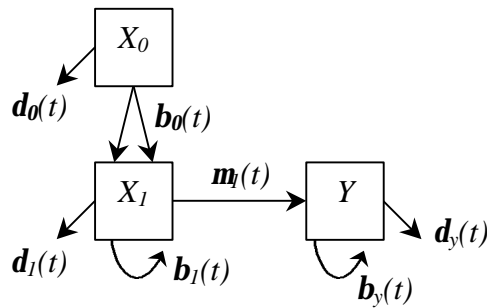


Figure 4: Modified CGD model that predicts results different from the Leroux et al. model. Table 4 lists all of the developmental rates associated with this particular CGD model.

The set of parameter values used to compare the two models is shown in Table 4.

Table 4: *Parameters Used and Estimates Obtained Among the Two Models*

	Leroux et al. Model		CGD Model		
	X	Y	X_0	X_1	Y
Initial number of cells (time $t=0$)	1.68×10^5	0	1.68×10^5	0	0
Birth rate, \mathbf{b}	0.4	0.4	0.4	0.4	0.4
Death rate, \mathbf{d}	0.01	0.01	0.01	0.4	0.01
Transformation rate, \mathbf{m}	0.15	--	0	0.5174	--
Expected number of cells (time $t=5$)	5.58×10^5	6.23×10^5	2.16×10^4	6.69×10^4	6.23×10^5
Total number of cells	1.181×10^6		7.115×10^5		

As before, both the Leroux et al. model and the simple CGD model (Figure 4) start with the same initial conditions. The type- X (Leroux et al. model) and type- X_0 (CGD model) subpopulations each consist of 1.68×10^8 cells at time $t = 0$ while the remaining states start with a cell population of zero. In this example, the transformation rate for the X_0 state is set equal to zero; thus, only cells from the X_1 state migrate to the Y state. The variance and covariance (i.e., the second moments) of each random variable is also set equal to zero at time $t = 0$. Each model is run for a total of five time units. It is noted that the only changes made to the CGD model are setting \mathbf{m}_0 to zero and

increasing d_1 and m_1 . Nevertheless, the results are quite different from those of the Leroux et al. model.

The expected value of type- Y cells for both models agree at time $t = 5$, as seen in Figure 5, but differ slightly at other time points.

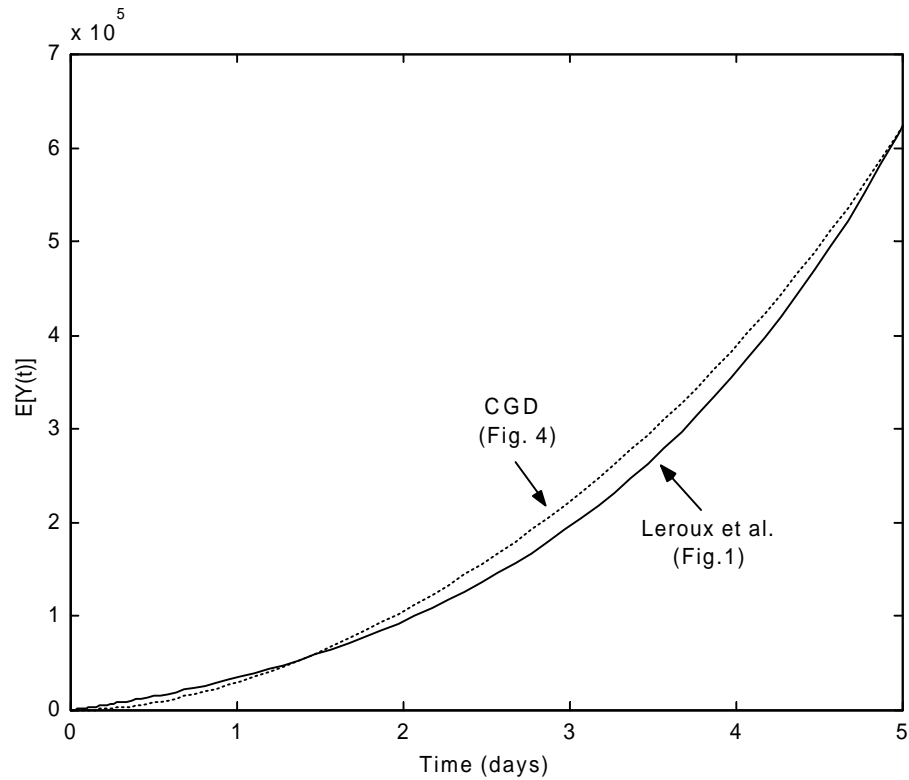


Figure 5: Expected number of type- Y cells as predicted by the Leroux et al. model and the CGD model using parameters in Table 4.

This implies that the CGD model can indeed be tailored to produce the same number of committed (type- Y) cells as the Leroux et al. model. However, Figure 6 reveals that the models produce different expected numbers of uncommitted (type- X) cells.

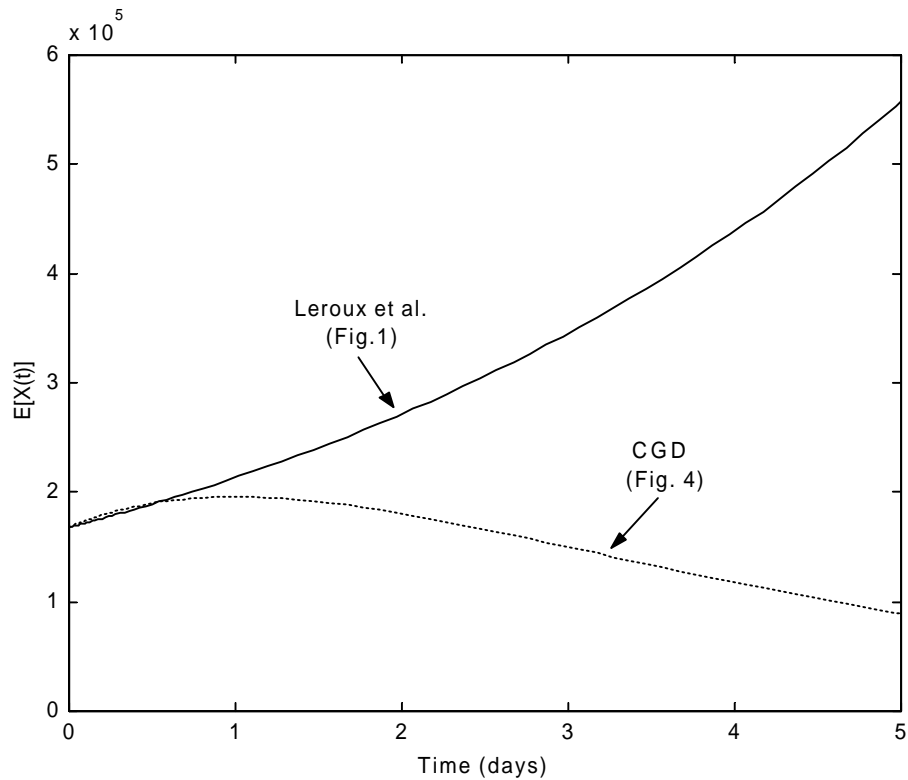


Figure 6: Expected number of type- X cells as predicted by the Leroux et al. model and the CGD model using parameters in Table 4.

As seen from Table 4, both models start out with the same number of uncommitted cells and the birth rates in each type- X population are the same. However, due to the structure of the CGD model, the type- X_1 cells are able to have higher death and transformation rates. Biologically, this means that all of the committed (type- Y) cells have type- X_1 precursor cells and that the population of uncommitted cells will eventually die out. This ultimately results in a stable population of committed cells at greater times, unlike the Leroux et al. model that will continue to grow.

Provided actual cell size is consistent throughout development, the expected final size of the tissue/organ is simply computed as the total number of cells present in the system at time $t = 5$. The total number of cells, which is defined to be the sum of all expectations of type- X and type- Y cells, present in the Leroux et al. model exceeds the number of cells present in the CGD model. This implies that, for sufficiently large time, the size of the tissue/organ might become unrealistically large. It is also important to note that in order to reduce the number of committed cells in the Leroux et al. model, there are a few options available:

- (i) reduce the initial number of type- X cells
- (ii) reduce the birth rate of the type- X and/or type- Y cells
- (iii) increase the death rate of the type- X and/or type- Y cells
- (iv) use more complicated time-varying rates

In each case, the ability to accurately reflect the biological dynamics of the development process is compromised. If option (i) is pursued, the experimental data and the mathematical model may not agree initially. If option (ii) or (iii) is incorporated, the cellular kinetics of the system may not be modeled accurately. In any event, the end result is that the mathematical model does not realistically duplicate the biological dynamics of the system. Option (iv) requires significantly more knowledge of the time-varying nature of the developmental rates in the system.

On the other hand, the CGD model can be amended to incorporate all of the biological dynamics of the developmental process with time-constant parameters while also yielding a feasible number of cells that will ultimately dictate the expected final

size of the tissue/organ. Even if the birth, death and/or transformation rates are reduced/increased in one state of the CGD model, a different state may be amended to reflect a different aspect of the system dynamics. In essence, the concept of homeostasis can be incorporated into the CGD model while still yielding a biologically feasible prediction at each time step.

Chapter 3: Controlled Growth and Differentiation Model Applications

Biologically-based mathematical models are developed based on the observations recorded by scientists. The models are usually validated against experimental data (when available) and then used to predict the results of various dynamical processes. The information gained from a biological model can also be used to assist in the judicious design of laboratory experiments, thereby reducing the unnecessary loss of valuable time and resources. The ultimate objective of the CGD model is to provide a more biologically realistic model for describing the dynamic process of organogenesis. As a step toward this goal, this chapter explores two applications for the CGD model.

The first application is a spermatogenesis model. Section 3.1 discusses the fundamental dynamics of sperm cell production. In Section 3.2 a CGD model is formulated that mathematically describes the process of spermatocytogenesis. We then implement time lags into the system of ordinary differential equations to more closely mimic the natural biological process of germ cell development.

The second application is a demonstration of how the spermatocytogenesis model can be used to model hormesis. The developmental rates for the spermatocytogenesis CGD model are changed from constant rates to dose-dependent rates. Thus for a given time, a dose-response curve is generated for an agent that produces a hormetic effect.

3.1 Spermatogenesis

The process in which nonspecialized stem cells mature and develop into sperm cells is spermatogenesis. During this developmental period, the cell progresses through several stages. The three major classes of cell types are spermatogonia, spermatocytes and spermatids [50]. The seminiferous tubule is composed of billions of connected seminiferous epithelium cells and is the primary site of spermatozoa production. Seminiferous epithelium cells are made up of peritubular cells on the outside and Sertoli and cells on the inside. Sertoli cells are tightly packed along the walls of the seminiferous tubule and function to support cells during spermatogenesis. The Sertoli cells are responsible for providing nourishment to the developing cells and directing the cell development throughout spermatogenesis. The unorthodox tree-like structure of the Sertoli cell allows for an intimate relationship between the “giver” (Sertoli cell) and the “taker” (developing germ cell) at all stages of development. The shape of the Sertoli cell is continuously changing. This allows the Sertoli cell to efficiently assist the developing cells through its maturation. Throughout spermatogenesis, Sertoli cells

support roughly 15 to 20 cells. Figure 7 shows the relationship between the Sertoli cell and developing cells.



Figure 7: The physical relationship of the Sertoli cell and developing cells. (Figure by Dr. L. Russel, Southern Illinois University School of Medicine [51])

3.1.1 Cellular Dynamics

During germ cell development, there are three major phases: spermatocytogenesis, meiosis and spermiogenesis. Cells in the earliest stage of spermatogenesis are in the spermatocytogenesis phase and are referred to as spermatogonia. These immature germ cells originate from stem cells that have undergone asymmetric division: a process where a stem cell divides and produces another stem cell (to maintain the original stem cell population) and an immature specialized cell. In this case, the future specialized cells are spermatogonia. Spermatogonia reside primarily at the base of the seminiferous tubule. The immature germ cell attaches itself to the Sertoli cell, its source of nutrition. As the cell continues through the developmental process, it migrates in an upward direction along the outer boundary of the Sertoli cell [50].

The second stage of spermatogenesis consists of spermatocytes that are in the meiosis phase of development. These cells are commonly classified as either primary spermatocytes (“first sperm cell”) or secondary spermatocytes (“second sperm cell”) [50]. By increasing the amount of cytoplasm, a spermatogonium develops into a primary spermatocyte. During meiosis I, the primary spermatocyte divides and gives rise to two secondary spermatocytes. Each secondary spermatocyte has 23 chromosomes. Secondary spermatocytes are haploid since each new nucleus only contains a single set of chromosomes. During reproduction, these 23 chromosomes combine with the 23 chromosomes of the female germ cell, the egg, producing a zygote,

which eventually develops into a fetus. Secondary spermatocytes proceed to the meiosis II phase producing two spermatids, each maintaining 23 chromosomes [52].

At this point of spermatogenesis, the spermiogenesis phase has started. No cell division occurs in this phase. Cells in this phase exist as both round spermatids and elongated spermatids. Although the physical shape of the cell changes, no significant molecular changes occur; hence, all spermatids (round and elongated) are classified together. As maturation continues, the round spermatid becomes oval in shape and develops a tail, resulting in an elongated spermatid. The Sertoli cell creates a deep indentation for the elongated spermatid to continue to develop. As the elongated spermatid matures with time into a sperm cell, the Sertoli cell carefully pushes the cell to the center of the seminiferous tubule. At this point of spermatogenesis the mature sperm cell is carried from the center of the seminiferous tubule to the epididymis. The epididymis, which is located in the testes, stores the mature sperm until it is ready to exit the male body [50].

3.1.2 A Mathematical Model for Spermatogenesis

The spermatocytogenesis phase of spermatogenesis produces seven different spermatogonia cell types. The process starts off with type- A cells. The stem cell, or type- A_0 cell, replicates periodically producing one stem cell and one type- A_1 daughter cell. The type- A_1 cell matures into a type- A_2 cell. This procedure of maturation continues as the cell develops into a type- A_3 cell and then type- A_4 cell. Type- A_4 cells

eventually enter an intermediate state and are thus classified as intermediate cells. After a specified amount of time, the spermatogonia cell leaves the intermediate stage and becomes a type-*B* cell. The presence of a type-*B* spermatogonia cell signifies the end of spermatocytogenesis. Type-*B* cells go through the first phase of meiosis, producing two preleptotene cells. preleptotene cells give rise to leptotene, zygotene, pachytene and diplotene cells, respectively. Meiosis is concluded with second degree spermatocytes dividing to produce two type 1 spermatids. These cells are simply classified by number (type1 through type 19 spermatids) since no further molecular changes occur.

A basic mathematical formulation of the biological process described above utilizing the CGD model would obviously involve several compartments. From the initial stem cell division to the final stage of spermatids is a total of 14 developmental steps, provided all spermatids are grouped together as one cell type (since no molecular changes are identified during spermiogenesis). This can be seen clearly in Figure 8.

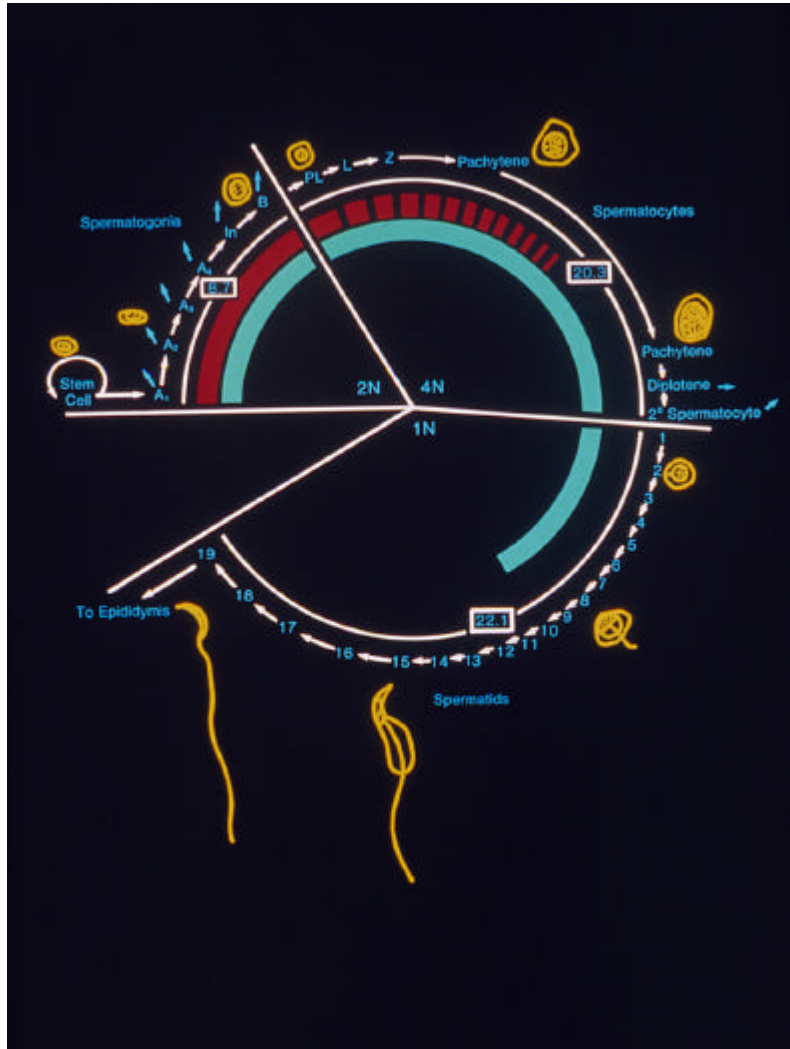


Figure 8: A schematic diagram of spermatogenesis. (Figure by Peter Working).

According to equation (2.23) (applicable only to stochastic systems) this would yield a system of 133 ordinary differential equations for the CGD model. To reduce the number of equations, a simplifying statistical assumption is made. The CGD model is

developed by treating spermatocytogenesis as a stochastic process while treating meiosis and spermiogenesis as deterministic. This assumption is made because the early stages of cell development, spermatocytogenesis, is most likely to have a probabilistic nature. For example, consider the case of over production of mature sperm cells. One cell has the potential to divide and produce hundreds of mature germ cells. In an effort to return the system to a state of equilibrium, the body will send out chemical signals to the cells. Thus, to rectify the problem of excess sperm, apoptosis (programmed cell death) may occur in the earliest stages of cell development. This will bring the system back to a state of biological equilibrium quicker than if apoptosis occurred in later phases of development (meiosis or spermiogenesis). Treating spermatocytogenesis as a stochastic process yields seven first moment equations for the CGD model. Figure 9 gives a visual description of a CGD model for spermatocytogenesis.

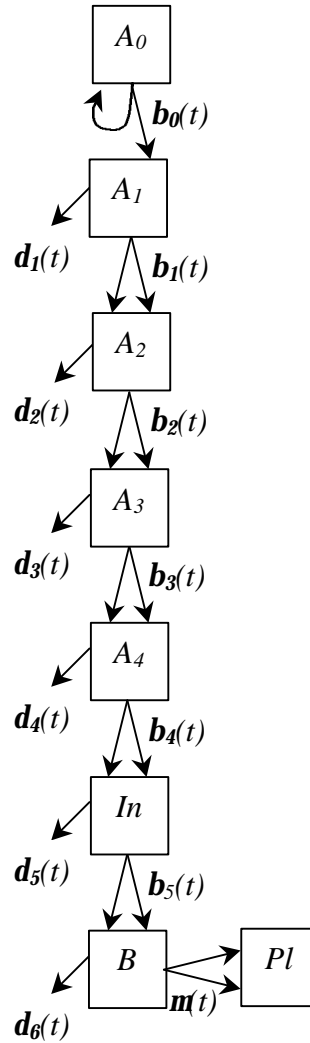


Figure 9: Schematic diagram of CGD model for spermatocytogenesis.

To determine the remaining equations for spermatocytogenesis, we let $n=7$ in equation (2.23). Thus, there are a total of 35 equations for the spermatocytogenesis CGD model. Add to those equations the seven equations from meiosis and spermiogenesis and the grand total yields a system of 42 ordinary differential equations. Thus, the system has been reduced by half its original size.

However, the modeling does not stop there. Information on immature germ cell dynamics indicates that the immature sperm cells progress through the various stages of development on a very precise and carefully measured schedule. For example, during normal sperm production the stem cell will divide approximately every 309.6 hours (dialogue with Dr. Chapin). Once the daughter cell enters the type- A_1 stage, it remains there for a given period of time. The cell “rests” in a particular stage for a prescribed amount of time until it has matured and is ready to proceed to the next stage, i.e., there is a delay in the transformation from one stage to the next. Figure 10 shows the amounts of time cells spend in each respective stage [53].

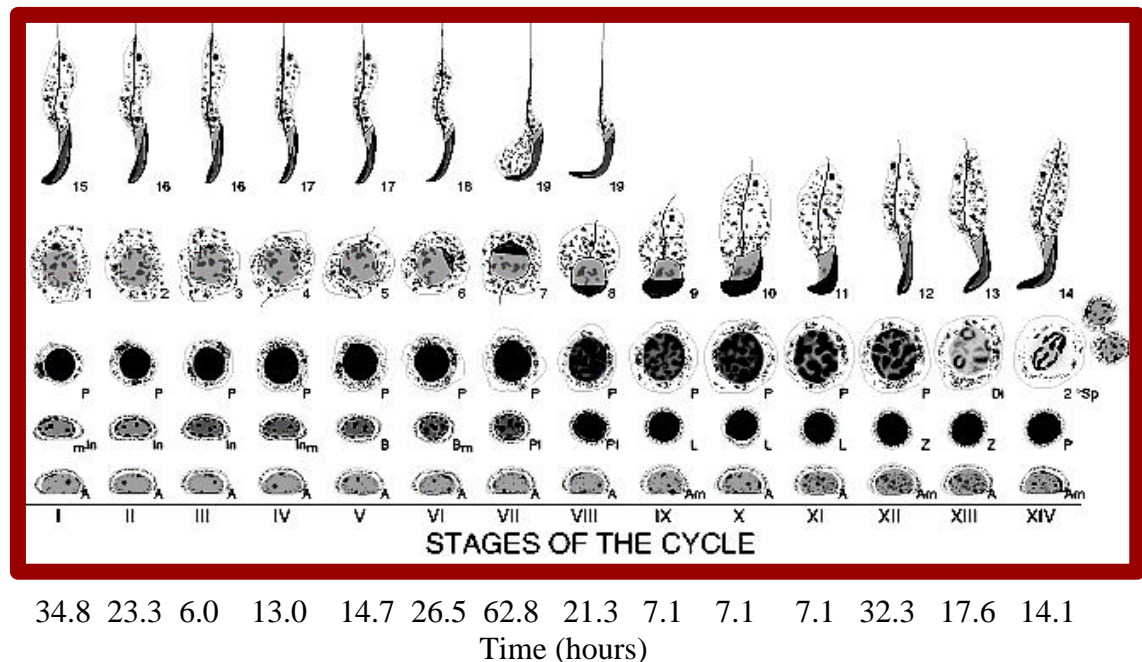


Figure 10: Cell Cycle with respect to time.

The Roman numbers in the figure indicate the particular developmental phase of a cell while the Arabic numbers indicate the duration of the stages in hours. Thus, in developing the CGD model for spermatocytogenesis, time delays are incorporated into the system of ordinary differential equations giving rise to a system of delay differential equations. Although the time lags are put into the system based upon the information in Figure 10, we note that after the system reaches steady state the lags are immaterial. The system of 42 delay differential equations describing spermatogenesis, where spermatocytogenesis is treated as stochastic and meiosis and spermiogenesis are treated as deterministic, is given below:

$$\frac{d}{dt}E[A_0(t)] = 0 \quad (3.1)$$

$$\frac{d}{dt}E[A_1(t)] = \mathbf{b}_0E[A_0(t - 309.6)] - \mathbf{b}_1E[A_1(t - 174.7)] \quad (3.2)$$

$$\frac{d}{dt}E[A_2(t)] = 2\mathbf{b}_1E[A_1(t - 174.7)] - \mathbf{b}_2E[A_2(t - 46.5)] \quad (3.3)$$

$$\frac{d}{dt}E[A_3(t)] = 2\mathbf{b}_2E[A_2(t - 46.5)] - \mathbf{b}_3E[A_3(t - 31.7)] \quad (3.4)$$

$$\frac{d}{dt}E[A_4(t)] = 2\mathbf{b}_3E[A_3(t - 31.7)] - \mathbf{b}_4E[A_4(t - 34.8)] \quad (3.5)$$

$$\frac{d}{dt}E[In(t)] = 2\mathbf{b}_4E[A_4(t - 34.8)] - \mathbf{b}_5E[In(t - 42.3)] \quad (3.6)$$

$$\frac{d}{dt}E[B(t)] = 2\mathbf{b}_5E[In(t - 42.3)] - \mathbf{m}E[B(t - 41.2)]. \quad (3.7)$$

$$\frac{d}{dt}E[Pl(t)] = 2\mathbf{m}E[B(t - 41.2)] - \mathbf{b}_6E[Pl(t - 84.1)] \quad (3.8)$$

$$\frac{d}{dt}E[L(t)] = \mathbf{b}_6E[B(t-84.1)] - \mathbf{b}_7E[L(t-21.3)] \quad (3.9)$$

$$\frac{d}{dt}E[Z(t)] = \mathbf{b}_7E[L(t-21.3)] - \mathbf{b}_8E[Z(t-49.9)] \quad (3.10)$$

$$\frac{d}{dt}E[P(t)] = \mathbf{b}_8E[Z(t-49.9)] - \mathbf{b}_9E[P(t-270.1)] \quad (3.11)$$

$$\frac{d}{dt}E[Di(t)] = \mathbf{b}_9E[P(t-270.1)] - \mathbf{b}_{10}E[Di(t-17.6)] \quad (3.12)$$

$$\frac{d}{dt}E[II(t)] = 2\mathbf{b}_{10}E[Di(t-17.6)] - \mathbf{b}_{11}E[II(t-14.1)] \quad (3.13)$$

$$\frac{d}{dt}E[S_r(t)] = 2\mathbf{b}_{11}E[II(t-14.1)] \quad (3.14)$$

$$\frac{d}{dt}E[A_0^2(t)] = -2\mathbf{b}_0E[A_0^2(t)] + \mathbf{b}_0E[A_0(t)] \quad (3.15)$$

$$\begin{aligned} \frac{d}{dt}E[A_1^2(t)] &= -2\mathbf{b}_1E[A_1^2(t-174.7)] + \mathbf{b}_1E[A_1(t-174.7)] \\ &\quad + 4\mathbf{b}_0E[A_0(t-309.6)] + 4\mathbf{b}_0E[A_0(t-309.6)A_1(t-174.7)] \end{aligned} \quad (3.16)$$

$$\begin{aligned} \frac{d}{dt}E[A_2^2(t)] &= -2(\mathbf{b}_2 + \mathbf{d}_2)E[A_2^2(t-46.5)] + (\mathbf{b}_2 + \mathbf{d}_2)E[A_2(t-46.5)] \\ &\quad + 4\mathbf{b}_1E[A_1(t-174.7)] + 4\mathbf{b}_1E[A_1(t-174.7)A_2(t-46.5)] \end{aligned} \quad (3.17)$$

$$\begin{aligned} \frac{d}{dt}E[A_3^2(t)] &= -2\mathbf{b}_3E[A_3^2(t-31.7)] + \mathbf{b}_3E[A_3(t-31.7)] \\ &\quad + 4\mathbf{b}_2E[A_2(t-46.5)] + 4\mathbf{b}_2E[A_2(t-46.5)A_3(t-31.7)] \end{aligned} \quad (3.18)$$

$$\begin{aligned} \frac{d}{dt}E[A_4^2(t)] &= -2\mathbf{b}_4E[A_4^2(t-34.8)] + \mathbf{b}_4E[A_4(t-34.8)] \\ &\quad + 4\mathbf{b}_3E[A_3(t-31.7)] + 4\mathbf{b}_3E[A_3(t-31.7)A_4(t-34.8)] \end{aligned} \quad (3.19)$$

$$\begin{aligned} \frac{d}{dt} E[In^2(t)] &= -2\mathbf{b}_5 E[In^2(t-42.3)] + \mathbf{b}_5 E[In(t-42.3)] \\ &\quad + 4\mathbf{b}_4 E[A_4(t-34.8)] + 4\mathbf{b}_4 E[A_4(t-34.8)In(t-42.3)] \end{aligned} \quad (3.20)$$

$$\begin{aligned} \frac{d}{dt} E[B^2(t)] &= -2\mathbf{m} E[B^2(t-41.2)] + \mathbf{m} E[B(t-41.2)] \\ &\quad + 4\mathbf{b}_5 E[In(t-42.3)] + 4\mathbf{b}_5 E[In(t-42.3)B(t-41.2)] \end{aligned} \quad (3.21)$$

$$\begin{aligned} \frac{d}{dt} E[A_0(t)A_1(t)] &= -2\mathbf{b}_0 E[A_0(t-309.6)] + 2\mathbf{b}_0 E[A_0^2(t-309.6)] \\ &\quad - (\mathbf{b}_0 + \mathbf{b}_1) E[A_0(t-309.6)A_1(t-174.7)] \end{aligned} \quad (3.22)$$

$$\begin{aligned} \frac{d}{dt} E[A_1(t)A_2(t)] &= -2\mathbf{b}_1 E[A_1(t-174.7)] + 2\mathbf{b}_1 E[A_1^2(t-174.7)] \\ &\quad + 2\mathbf{b}_0 E[A_0(t-309.6)A_2(t-46.5)] \\ &\quad - (\mathbf{b}_1 + \mathbf{b}_2 + \mathbf{d}_2) E[A_1(t-174.7)A_2(t-46.5)] \end{aligned} \quad (3.23)$$

$$\begin{aligned} \frac{d}{dt} E[A_2(t)A_3(t)] &= -2\mathbf{b}_2 E[A_2(t-46.5)] + 2\mathbf{b}_2 E[A_2^2(t-46.5)] \\ &\quad + 2\mathbf{b}_1 E[A_1(t-174.7)A_3(t-31.7)] \\ &\quad - (\mathbf{b}_2 + \mathbf{d}_2 + \mathbf{b}_3) E[A_2(t-46.5)A_3(t-31.7)] \end{aligned} \quad (3.24)$$

$$\begin{aligned} \frac{d}{dt} E[A_3(t)A_4(t)] &= -2\mathbf{b}_3 E[A_3(t-31.7)] + 2\mathbf{b}_3 E[A_3^2(t-31.7)] \\ &\quad + 2\mathbf{b}_2 E[A_2(t-46.5)A_4(t-34.8)] \\ &\quad - (\mathbf{b}_3 + \mathbf{b}_4) E[A_3(t-31.7)A_4(t-34.8)] \end{aligned} \quad (3.25)$$

$$\begin{aligned} \frac{d}{dt} E[A_4(t)In(t)] &= -2\mathbf{b}_4 E[A_4(t-34.8)] + 2\mathbf{b}_4 E[A_4^2(t-34.8)] \\ &\quad + 2\mathbf{b}_3 E[A_3(t-31.7)In(t-42.3)] \\ &\quad - (\mathbf{b}_4 + \mathbf{b}_5) E[A_4(t-34.8)In(t-42.3)] \end{aligned} \quad (3.26)$$

$$\begin{aligned} \frac{d}{dt} E[In(t)B(t)] &= -2\mathbf{b}_5 E[In(t-42.3)] + 2\mathbf{b}_5 E[In(t-42.3)] \\ &\quad + 2\mathbf{b}_4 E[A_4(t-34.8)B(t-41.2)] \\ &\quad - (\mathbf{b}_5 + \mathbf{m}) E[In(t-42.3)B(t-41.2)] \end{aligned} \quad (3.27)$$

$$\begin{aligned} \frac{d}{dt} E[A_0(t)A_2(t)] &= -(\mathbf{b}_0 + \mathbf{b}_2 + \mathbf{d}_2) E[A_0(t-309.6)A_2(t-46.5)] \\ &\quad + 2\mathbf{b}_1 E[A_0(t-309.6)A_1(t-174.7)] \end{aligned} \quad (3.28)$$

$$\begin{aligned} \frac{d}{dt} E[A_0(t)A_3(t)] &= -(\mathbf{b}_0 + \mathbf{b}_3) E[A_0(t-309.6)A_3(t-31.7)] \\ &\quad + 2\mathbf{b}_2 E[A_0(t-309.6)A_2(t-46.5)] \end{aligned} \quad (3.29)$$

$$\begin{aligned} \frac{d}{dt} E[A_0(t)A_4(t)] &= -(\mathbf{b}_0 + \mathbf{b}_4) E[A_0(t-309.6)A_4(t-34.8)] \\ &\quad + 2\mathbf{b}_3 E[A_0(t-309.6)A_3(t-31.7)] \end{aligned} \quad (3.30)$$

$$\begin{aligned} \frac{d}{dt} E[A_0(t)In(t)] &= -(\mathbf{b}_0 + \mathbf{b}_5) E[A_0(t-309.6)In(t-42.3)] \\ &\quad + 2\mathbf{b}_4 E[A_0(t-309.6)A_4(t-34.8)] \end{aligned} \quad (3.31)$$

$$\begin{aligned} \frac{d}{dt} E[A_0(t)B(t)] &= -(\mathbf{b}_0 + \mathbf{m}) E[A_0(t-309.6)B(t-41.2)] \\ &\quad + 2\mathbf{b}_5 E[A_0(t-309.6)In(t-42.3)] \end{aligned} \quad (3.32)$$

$$\begin{aligned} \frac{d}{dt} E[A_1(t)A_3(t)] &= -(\mathbf{b}_1 + \mathbf{b}_3) E[A_1(t-174.7)A_3(t-31.7)] \\ &\quad + 2\mathbf{b}_0 E[A_0(t-309.6)A_3(t-31.7)] \\ &\quad + 2\mathbf{b}_2 E[A_1(t-174.7)A_2(t-46.5)] \end{aligned} \quad (3.33)$$

$$\begin{aligned} \frac{d}{dt} E[A_1(t)A_4(t)] &= -(\mathbf{b}_1 + \mathbf{b}_4) E[A_1(t-174.7)A_4(t-34.8)] \\ &\quad + 2\mathbf{b}_0 E[A_0(t-309.6)A_4(t-34.8)] \\ &\quad + 2\mathbf{b}_3 E[A_1(t-174.7)A_3(t-31.7)] \end{aligned} \quad (3.34)$$

$$\begin{aligned} \frac{d}{dt} E[A_1(t)In(t)] &= -(\mathbf{b}_1 + \mathbf{b}_5) E[A_1(t-174.7)In(t-42.3)] \\ &\quad + 2\mathbf{b}_0 E[A_0(t-309.6)In(t-42.3)] \\ &\quad + 2\mathbf{b}_4 E[A_1(t-174.7)A_4(t-34.8)] \end{aligned} \quad (3.35)$$

$$\begin{aligned}
\frac{d}{dt} E[A_1(t)B(t)] &= -(\mathbf{b}_1 + \mathbf{m}) E[A_1(t-174.7)B(t-41.2)] \\
&\quad + 2\mathbf{b}_0 E[A_0(t-309.6)B(t-41.2)] \\
&\quad + 2\mathbf{b}_5 E[A_1(t-174.7)In(t-42.3)]
\end{aligned} \tag{3.36}$$

$$\begin{aligned}
\frac{d}{dt} E[A_2(t)A_4(t)] &= -(\mathbf{b}_2 + \mathbf{d}_2 + \mathbf{b}_4) E[A_2(t-46.5)A_4(t-34.8)] \\
&\quad + 2\mathbf{b}_1 E[A_1(t-174.7)A_4(t-34.8)] \\
&\quad + 2\mathbf{b}_3 E[A_2(t-46.5)A_3(t-31.7)]
\end{aligned} \tag{3.37}$$

$$\begin{aligned}
\frac{d}{dt} E[A_2(t)In(t)] &= -(\mathbf{b}_2 + \mathbf{d}_2 + \mathbf{b}_5) E[A_2(t-46.5)In(t)] \\
&\quad + 2\mathbf{b}_1 E[A_1(t-174.7)In(t-42.3)] \\
&\quad + 2\mathbf{b}_4 E[A_2(t-46.5)A_4(t-34.8)]
\end{aligned} \tag{3.38}$$

$$\begin{aligned}
\frac{d}{dt} E[A_2(t)B(t)] &= -(\mathbf{b}_2 + \mathbf{d}_2 + \mathbf{m}) E[A_2(t-46.5)B(t)] \\
&\quad + 2\mathbf{b}_1 E[A_1(t-174.7)B(t-41.2)] \\
&\quad + 2\mathbf{b}_5 E[A_2(t-46.5)In(t-42.3)]
\end{aligned} \tag{3.39}$$

$$\begin{aligned}
\frac{d}{dt} E[A_3(t)In(t)] &= -(\mathbf{b}_3 + \mathbf{b}_5) E[A_3(t-31.7)In(t-42.3)] \\
&\quad + 2\mathbf{b}_2 E[A_2(t-46.5)In(t-42.3)] \\
&\quad + 2\mathbf{b}_4 E[A_3(t-31.7)A_4(t-34.8)]
\end{aligned} \tag{3.40}$$

$$\begin{aligned}
\frac{d}{dt} E[A_3(t)B(t)] &= -(\mathbf{b}_3 + \mathbf{m}) E[A_3(t-31.7)B(t-41.2)] \\
&\quad + 2\mathbf{b}_2 E[A_2(t-46.5)B(t-41.2)] \\
&\quad + 2\mathbf{b}_5 E[A_3(t-31.7)In(t-42.3)]
\end{aligned} \tag{3.41}$$

$$\begin{aligned}
\frac{d}{dt} E[A_4(t)B(t)] &= -(\mathbf{b}_4 + \mathbf{m}) E[A_4(t-34.8)B(t-41.2)] \\
&\quad + 2\mathbf{b}_3 E[A_3(t-31.7)B(t-41.2)] \\
&\quad + 2\mathbf{b}_5 E[A_4(t-34.8)In(t-42.3)]
\end{aligned} \tag{3.42}$$

Recall from Figure 9 that the CGD model can accommodate birth, death and transformation rates at each level of the model. However, the CGD model was coded in MatLab using primarily birth rates.

Initially a relatively random choice of developmental rates for the spermatocytogenesis CGD model was chosen. Table 5 gives the birth, death and transformation rates used in the first simulation of spermatocytogenesis.

Table 5: *First parameter set used in CGD model for spermatocytogenesis.*

Cell Stage	Birth Rates	Death Rates	Transformation Rates
A_0	$\frac{1}{309.6}$	0	--
A_1	$\frac{1}{174.7}$	0	--
A_2	$\frac{1}{46.5}$	0	--
A_3	$\frac{1}{31.7}$	0	--
A_4	$\frac{1}{34.8}$	0	--
<i>In</i>	$\frac{1}{42.3}$	0	--
<i>B</i>	--	0	$\frac{1}{41.2}$

The first parameter set produces a dynamic behavior that is shown in Figure 11.

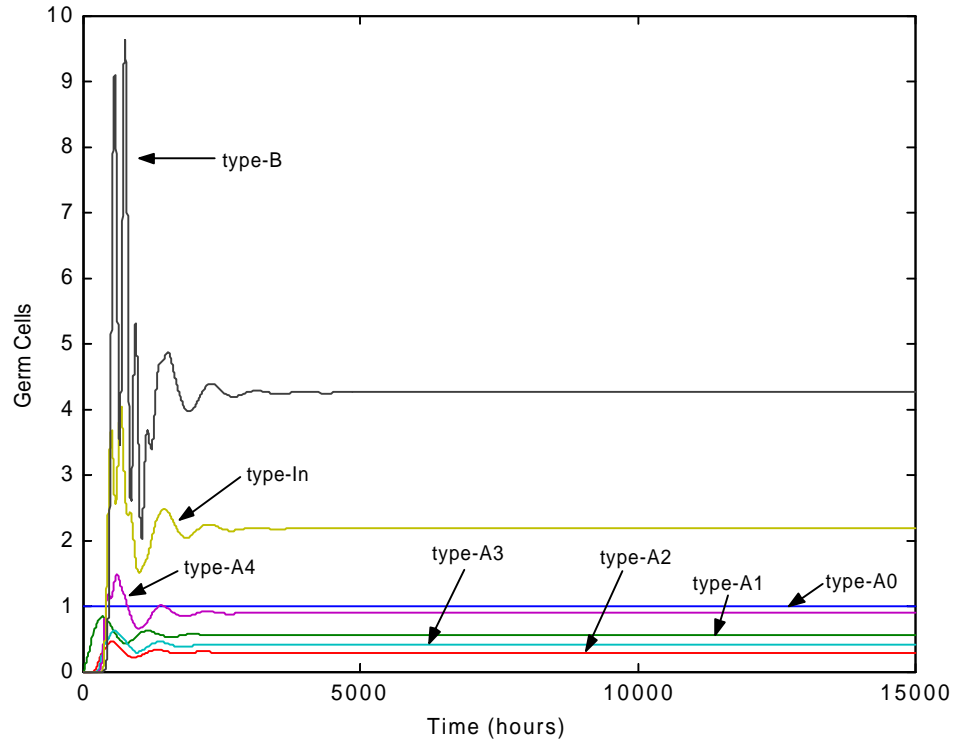


Figure 11: Chaotic response for spermatocytogenesis using the CGD model. Parameters used in this model are given in Table 5.

The activities of the system at early time points reflect a somewhat chaotic response. However, as time increases, the system reaches a steady state. In an effort to produce curves showing more favorable system dynamics, a second parameter set was chosen. Table 6 lists the developmental rates used for the second simulation.

Table 6: *Second parameter set used in CGD model for spermatocytogenesis.*

Cell Stage	Birth Rates	Death Rates	Transformation Rates
A_0	$\frac{1}{309.6}$	0	--
A_1	$\frac{1}{325.7}$	0	--
A_2	$\frac{1}{350.5}$	0	--
A_3	$\frac{1}{375.7}$	0	--
A_4	$\frac{1}{400.8}$	0	--
In	$\frac{1}{425.3}$	0	--
B	--	0	$\frac{1}{450.1}$

The second set of parameter values produces a smoother response, as seen in Figure 12.

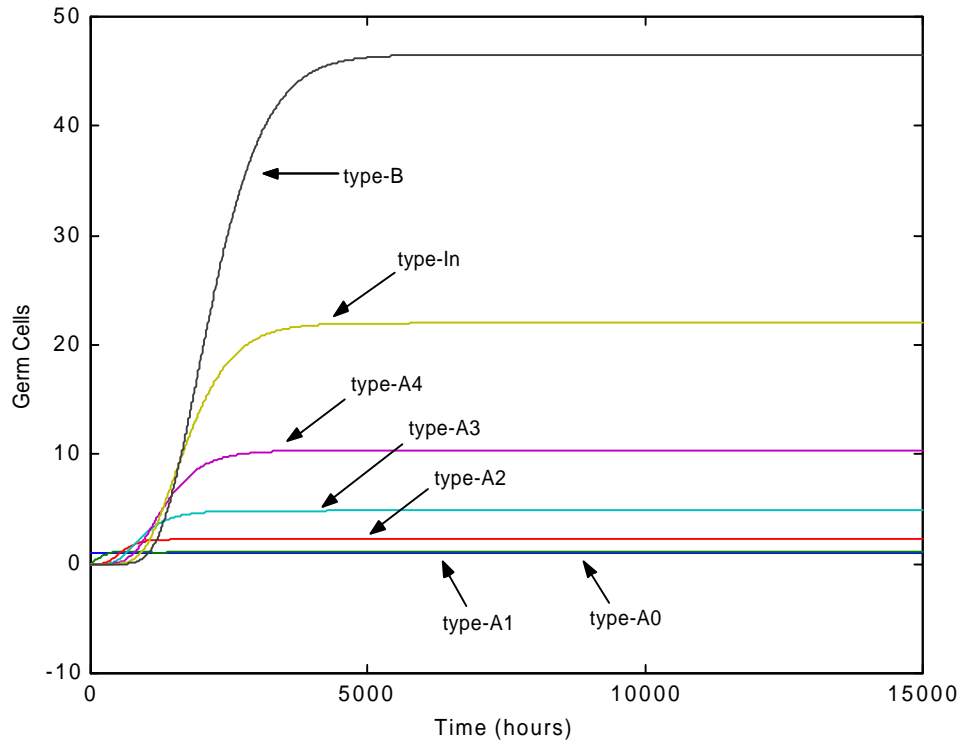


Figure 12: Smooth response for spermatocytogenesis using CGD model. Parameters used in this model are given in Table 6.

Not only does the system reach a steady state, but it does so in a less chaotic fashion relative to the parameters listed in Table 5. Also note that between $t=0$ hours and $t=1000$ hours, each cell is introduced into the system at a predetermined time (see Section 3.1.2 for details). This is the desired response for a biologically-based mathematical model with time delays. The stability analysis studies of this delay differential equation will be described in Chapter 4 of this thesis. Figure 13 is a plot of the sum of all the cells present during spermatocytogenesis over time using the

parameters from Table 6. The curve coincides with what is thought to be the normal developmental trend of germ cells in the testis [Chapin, personal communication #89].

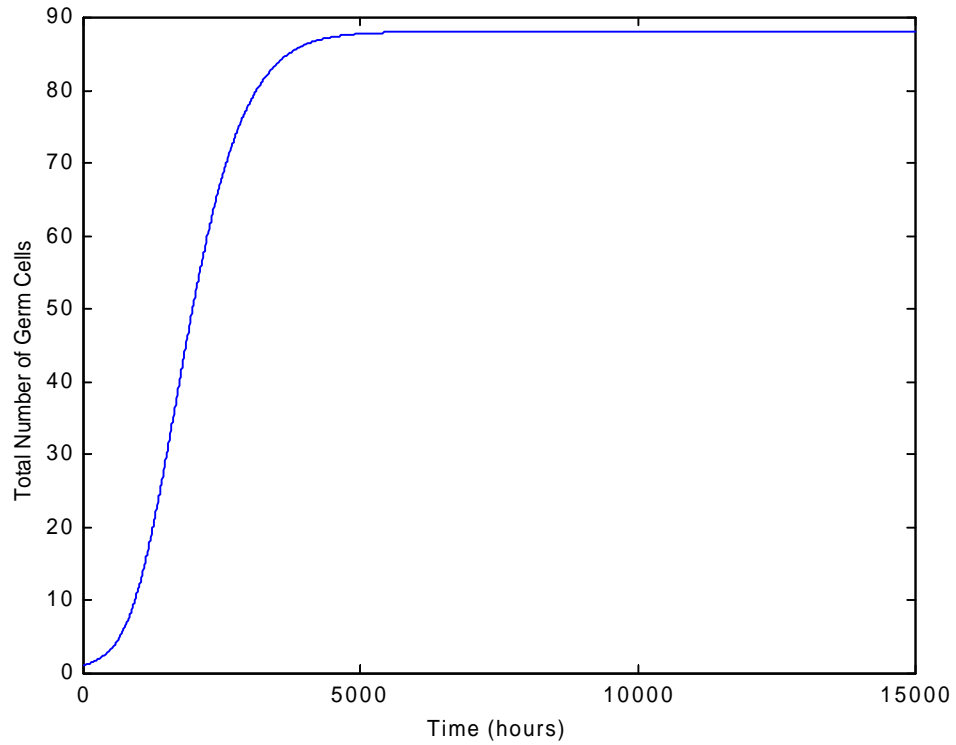


Figure 13: Total number of cells using spermatocytogenesis CGD model. Parameters used in this model are given in Table 6.

Although no known experimental data is available at the time to validate the model, the spermatocytogenesis CGD model is capable of duplicating various biologically realistic results.

3.2 Hormesis

Hormesis is often described as a dose-response relationship that is stimulatory at low doses, but inhibitory at a high dose [54]. The idea *that a little bit of a bad thing is good for you* summarizes the paradoxical essence of hormesis. Historically, risks of high environmental exposures have received the most scientific attention; thus drawing attention away from the lower end of the dose-response relationship. However, the scientific phenomenon of hormesis has refocused attention back to the effects of toxic agents in small doses.

3.2.1 Biological Responses that Cause Hormesis

Independent of its newly found fame, hormetic effects have been ubiquitous for quite some time. Johnson [55] considers several practical examples of hormesis in everyday life. The toxic and even lethal effect of alcohol consumption in high doses is by no means a novel scientific revelation. However, it was not noted until near the end of the 20th century that if red wine is consumed within moderation it can actually have positive health effects. Over the counter drugs are also substances that yield hormetic effects. The common household anti-inflammatory agent, aspirin, was manufactured with the primary intent of having positive health effects if taken in a reasonable dosage. But it is common knowledge that the same healing agent is capable of producing detrimental biological responses when taken in excess of the recommended dosage (i.e., “over-dosing”). Exercise can also be placed in a category where a dose-response

relationship has a hormetic effect. As Johnson and Bruunsgaard point out, research continues to support the perspective that low to moderate exercise has positive health effects. On the other hand, research also supports the claim that exercise can be hazardous to one's health. This would be a case of *too much of a good thing is bad for you*. Exercise that is prolonged or too strenuous can cause the immune system to behave counterproductively. Although it is not initially intuitive, stress is an element of life such that within reason, can be beneficial. But obviously too much stress can lead to serious health problems such as hypertension, ulcers or even death. However, stress within moderation has been scientifically proven to be "helpful" in acquiring an optimal quality of life. Typically, stress of this nature is often referred to as "challenges". In small doses, stress can have a positive outcome [55].

As noted, there are several examples of practical hormesis in everyday life. However, experimental biology continues to overlook hormetic possibilities when designing experiments that reflect only a linear dose-response relationship. These experiments have been criticized for not carefully considering hormesis at low doses. Existing models have been criticized for having dose spacing that is too large to accurately identify the possibility of hormesis [56]. This limitation in the study design can be resolved by administering more intermediate doses where hormesis is hypothesized to occur [57]. Incorporating lower end doses into the study design would improve the detection of toxic agents that produce hormetic effects.

The lack of hormesis identifiability can also be attributed to some of the assumptions made from the quantitative perspective. The traditional mathematical

models that are designed to produce dose-response curves only have the power to replicate linear responses. The assumption that low doses yield linear responses is clearly a flaw in the present mathematical models used [56]. In a manuscript by Calabrese and Baldwin [57], the authors cite several examples of reliable studies that support the claim that dose-response relationships do not necessarily follow a linear model at low doses. These studies continuously support the hormesis hypothesis, i.e., dose-response relationships follow a nonlinear model at low doses.

3.2.2 Modeling Hormesis

Most hormetic effects follow either a beta or a U-shaped (J-shaped) curve pattern, where the U-shaped dose-response curves are inversely comparable to the beta curve [58]. Endpoints for beta curves include growth, longevity, fecundity and weight gain. Conversely, examples of U-shaped curve endpoints are mutations, cancer incidence and birth-defects incidence [57]. From the quantitative perspective of hormesis, most mathematical models are not capable of predicting a hormetic effect due to the inherent assumption in the model that dictates a linear relationship between dose and biological responses at low doses. Models are needed that can predict nonlinear responses at low dose levels when a hormetic effect is present [54, 56]. Due to its properties and design, the CGD model is capable of predicting nonlinear responses at low doses, while also retaining the ability to predict linear responses. The modeler has control over the complexity, the amount of detail and the various developmental rates at each stage of

development in the CGD model. With the amount of modeling freedom that is inherent in the CGD model, nonlinear responses can be modeled that were once forced to be modeled as linear responses.

Since examples found in growth processes tend to conform to the beta-curve [59], we reconsider the spermatocytogenesis CGD model for modeling hormesis. Suppose we wish to model a toxic agent that is assumed to be administered in a dose amount from zero to one. It is assumed that the toxic agent only affects the cell population at specific stages of development. This concept is shown in the schematic diagram in Figure 14.

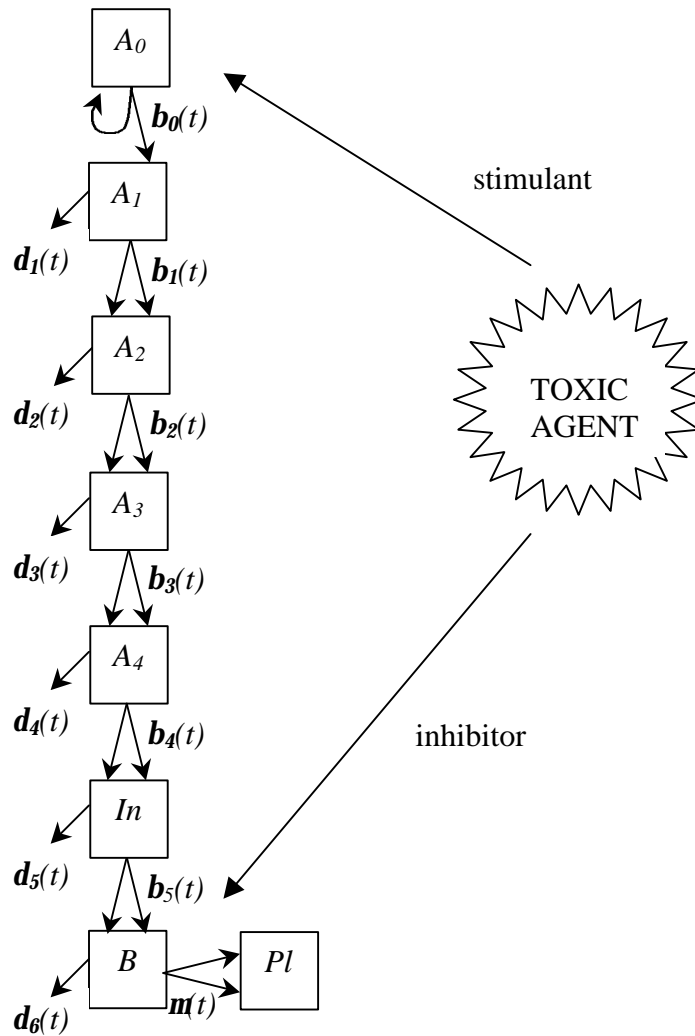


Figure 14: Schematic diagram of spermatocytogenesis CGD model for hormesis. A toxic agent acts as a stimulant at one stage of development while acting as an inhibitor at another stage.

Some researchers (e.g. [60]) support the perspective that certain agents act as a mitotic stimulator. Consider the case where the parent compound appears to promote growth at very low dose levels, but the same studies also show a decrease in the growth dynamics

at higher dose ranges. In this case, metabolites may actually cause a toxic effect and not the agent that was directly metabolized.

Several numerical simulations were performed to highlight the versatility of the CGD model and its ability to mimic hormetic effects. Dose-response relationships were attained using the CGD model by solving the system of delay differential equations at a specific time ($t=15000$) using dose-dependent developmental rates. The resulting plots show how dose affects the number of cells present in the system.

Since low doses of agents may enhance cellular promotion [57], a dose-dependent birth rate was formulated to be used in the spermatocytogenesis CGD model. In the first simulation, the birth rate of the first developmental stage was expressed as an increasing dose-dependent function. In all the simulations, the transformation rate was consistently expressed as a dose-dependent function. The other developmental rates remained constant. This set up corresponds to the toxic agent acting as a stimulant and inducing mitotic division. As can be seen from Figure 15, the total number of cells increases with dose. Table 7 lists the parameters that were used to generate the curve in Figure 15.

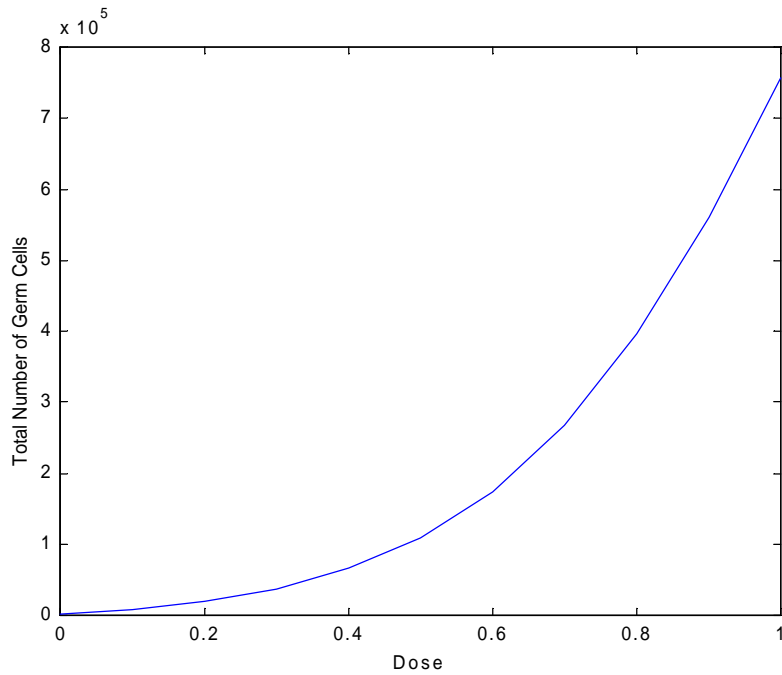


Figure 15: Dose-response relationship for spermatocytogenesis CGD model with a dose-dependent birth rate.

Table 7: Developmental rates used in CGD model for hormesis (d =dose).

Cell Stage	Birth Rates	Death Rates	Transformation Rates
A_0	$\frac{1}{309.6} * d$	0	--
A_1	$\frac{1}{225.7}$	0	--
A_2	$\frac{1}{90.5}$	0	--
A_3	$\frac{1}{75.7}$	0	--
A_4	$\frac{1}{60.8}$	0	--
In	$\frac{1}{80.3}$	0	--
B	--	0	$\frac{1}{450.1} e^{-3*d}$

Also, we incorporated a dose-dependent death rate in a later stage of the model. The second simulation allows the death rate of the last developmental stage to increase exponentially with dose. This corresponds to the toxic agent killing cells and thus inhibiting the growth of the tissue by reducing the total number of functioning cells. Figure 16 shows the dynamics of the system using the parameters in Table 8.

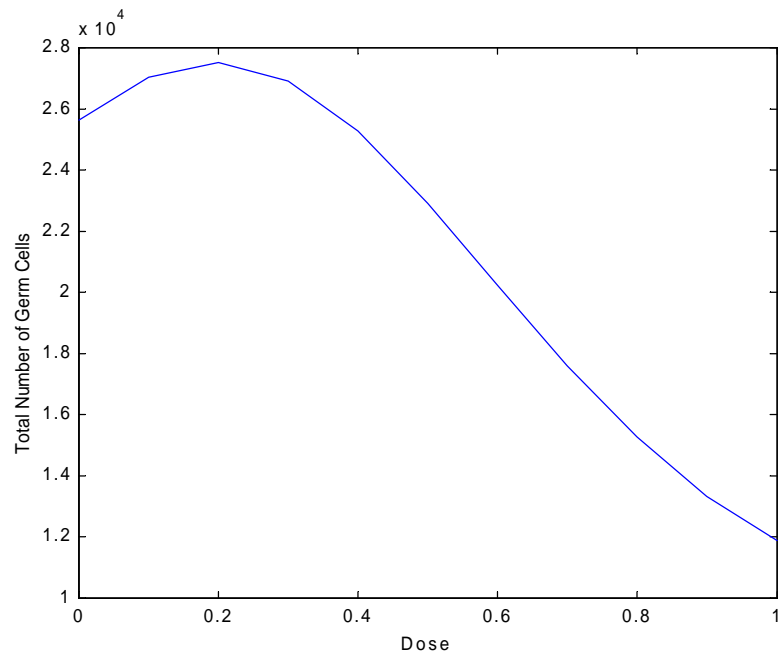


Figure 16: Dose-response relationship for spermatocytogenesis CGD model with a dose-dependent death rate.

Table 8: *Developmental rates used in CGD model for hormesis (d=dose).*

Cell Stage	Birth Rates	Death Rates	Transformation Rates
A_0	$\frac{1}{309.6}$	0	--
A_1	$\frac{1}{225.7}$	0	--
A_2	$\frac{1}{90.5}$	0	--
A_3	$\frac{1}{75.7}$	0	--
A_4	$\frac{1}{60.8}$	0	--
In	$\frac{1}{80.3}$	0	--
B	--	$\frac{1}{250} e^{2.2*d^2}$	$\frac{1}{450.1} e^{-3*d}$

The final simulation is a combination of the first two. The linear birth rate of the first developmental stage and the exponential death rate of the last developmental stage are incorporated into the spermatocytogenesis CGD model. The birth rate of the first developmental stage represents the early stimulation of the growth process by the toxic agent. The death rate of the last developmental stage represents the rapid rise in cell death as a function of dose. The result is a plot that indicates a slight hormetic effect (Figure 17). Table 9 lists all of the developmental rates used in the spermatocytogenesis CGD model to mimic a hormetic effect.

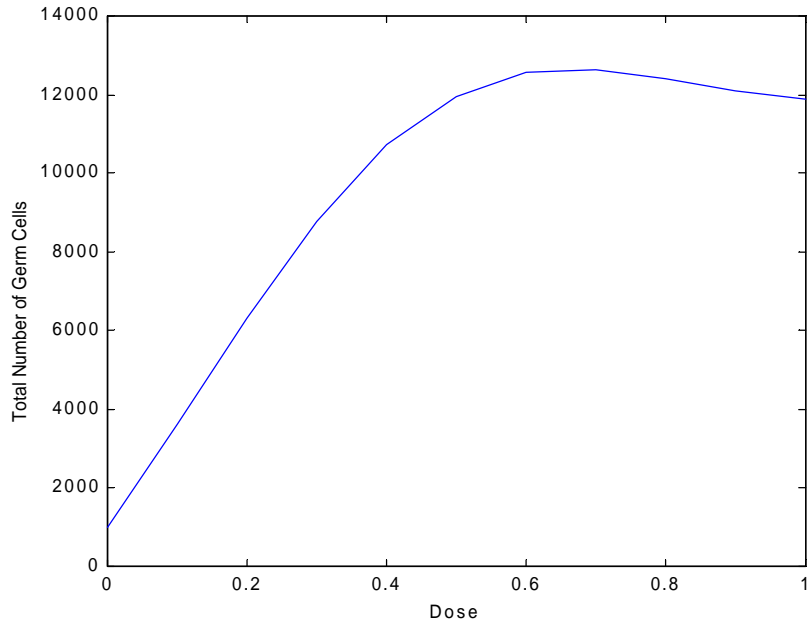


Figure 17: Beta curve generated from spermatocytogenesis CGD model. The parameters used in Table 9 yield a dose-response relationship with hormetic effect for the spermatocytogenesis CGD model.

Table 9: Developmental rates used in CGD model for hormesis (d =dose).

Cell Stage	Birth Rates	Death Rates	Transformation Rates
A_0	$\frac{1}{309.6} * d$	0	--
A_1	$\frac{1}{225.7}$	0	--
A_2	$\frac{1}{90.5}$	0	--
A_3	$\frac{1}{75.7}$	0	--
A_4	$\frac{1}{60.8}$	0	--
In	$\frac{1}{80.3}$	0	--
B	--	$\frac{1}{250} e^{2.2*d^2}$	$\frac{1}{450.1} e^{-3*d}$

As seen in Figure 17, the trend at low doses indicates an increase in cell number; however, the number of cells present in the system begin to decrease at higher doses. The resulting beta curve shows that at time $t = 15000$ hours, a hormetic effect occurs in the dose range 0 to 1. Initially, the hypothetical toxic agent acts as a mitotic stimulant and enhances the growth of the cell population. But at a critical dose value ($dose=0.6$) the slope of the response changes and begins to decrease.

The information gained from the beta curve in Figure 17 provides insightful information that is not detectable by all models. A U-shaped curve is also reproducible by the CGD model using the same type of logic for the choice of dose-dependent developmental rates.

Chapter 4: Theoretical Issues

This chapter investigates the existence, uniqueness and stability conditions for linear systems with multiple delays. Section 4.1 reformulates the CGD spermatocytogenesis model (developed Section 3.1). The existence and uniqueness of linear systems with delays, which are now well-known and are well documented in the literature, is presented in Section 4.2. A brief presentation on the stability theory for simple linear systems without delay is reviewed in Section 4.3. Section 4.4 describes stability analysis of linear delay differential equations using Lyapunov theory. In Section 4.5, stability conditions are applied to the spermatocytogenesis CGD model to determine the stability of the system.

4.1 Problem Reformulation

Recall that the first moments of the spermatocytogenesis CGD model are given by the following system of delay differential equations with discrete time delays:

$$\frac{d}{dt}E[A_0(t)] = 0 \quad (4.1)$$

$$\frac{d}{dt}E[A_1(t)] = \mathbf{b}_0E[A_0(t - 309.6)] - \mathbf{b}_1E[A_1(t - 174.7)] \quad (4.2)$$

$$\frac{d}{dt}E[A_2(t)] = 2\mathbf{b}_1E[A_1(t - 174.7)] - \mathbf{b}_2E[A_2(t - 46.5)] \quad (4.3)$$

$$\frac{d}{dt}E[A_3(t)] = 2\mathbf{b}_2E[A_2(t - 46.5)] - \mathbf{b}_3E[A_3(t - 31.7)] \quad (4.4)$$

$$\frac{d}{dt}E[A_4(t)] = 2\mathbf{b}_3E[A_3(t - 31.7)] - \mathbf{b}_4E[A_4(t - 34.8)] \quad (4.5)$$

$$\frac{d}{dt}E[In(t)] = 2\mathbf{b}_4E[A_4(t - 34.8)] - \mathbf{b}_5E[In(t - 42.3)] \quad (4.6)$$

$$\frac{d}{dt}E[B(t)] = 2\mathbf{b}_5E[In(t - 42.3)] - \mathbf{m}E[B(t - 41.2)]. \quad (4.7)$$

Let

$$x(t) = [E[A_0(t)], E[A_1(t)], E[A_2(t)], E[A_3(t)], E[A_4(t)], E[In(t)], E[B(t)]]^T \quad (4.8)$$

so that

$$\dot{x}(t) = \frac{d}{dt}[E[A_0(t)], E[A_1(t)], E[A_2(t)], E[A_3(t)], E[A_4(t)], E[In(t)], E[B(t)]]^T. \quad (4.9)$$

Define R to be the matrix of developmental rates associated with the various stages of spermatocytogenesis, i.e.,

4.2 Remarks on Existence and Uniqueness Results of Solutions of Hereditary Systems

A simple case of a functional differential equation is the differential-difference equation of the form

$$\dot{x}(t) = f(x(t), x(t-h), t) \quad (4.20)$$

where $h > 0$ is the delay and, for fixed t , $x(t) \in \mathbb{R}^n$. A moment of reflection indicates that in order to solve (4.20) for $t > t_0$ we must not only specify $x(t_0)$ but also the function $t \rightarrow x(t)$ on the interval $[t_0 - h, t_0]$. That is, let $\mathbf{f} \in C([-h, 0], \mathbb{R}^n)$ and consider

$$\dot{x}(t) = f(x(t), x(t-h), t) \quad (4.21)$$

with

$$x(t) = \mathbf{f}(t - t_0), t \in [t_0 - h, t_0]. \quad (4.22)$$

Then on the interval $[t_0, t_0 + h]$, equation (4.20) becomes

$$\dot{x}(t) = f(x(t), \mathbf{f}(t-h), t) \triangleq g(x(t), t), \quad (4.23)$$

since \mathbf{f} is a known function. If we assume that $f : \mathbb{R}^n \times \mathbb{R}^n \times \mathbb{R} \rightarrow \mathbb{R}^n$ is continuous and is Lipschitz with respect to the first argument, [49], the solution of $\dot{x} = g(x(t), t)$ exists on $[t_0, t_0 + h]$ and is unique. This gives us an initial condition for the solution on

$[t_0 + h, t_0 + 2h]$; and by simple induction arguments yield existence and uniqueness on $[t_0, t_1]$ for any $t_1 > t_0$.

From the above arguments, the function $t \rightarrow f(x(t), \mathbf{f}(t - t_0 - h), t)$ is continuous on $[t_0, t_0 + h]$ which yields that the solution starting at t_0 from the initial function \mathbf{f} , $x(t; \mathbf{f}, t_0)$, is of class C^1 on $(t_0, t_0 + h)$, hence on (t_0, t_1) . Moreover, if $f(\cdot)$ is k times continuously differentiable on $\mathbb{R}^n \times \mathbb{R}^n \times \mathbb{R}$ and $t_1 > t_0 + kh$, the solution $x(t; \mathbf{f}, t_0)$ will be of class C^2 on $(t_0 + h, t_1)$ and of class C^j on $(t_0 + (j-1)h, t_1)$, $j = 1, 2, \dots, k+1$. The fact that the regularity of the solution $x(t; \mathbf{f}, t_0)$ improves from interval to interval is a characteristic feature of retarded type differential-difference equations.

Next, we consider the general form of a linear system that involves a Lebesgue-Stieltjes integral of the form

$$\dot{x}(t) = \int_{-r}^0 [d_{\mathbf{q}} \mathbf{h}(t, \mathbf{q})] x(t + \mathbf{q}) \quad (4.24)$$

where $\mathbf{h}(t, \mathbf{q})$ is a matrix valued function and is assumed to be measurable in t and of bounded variation in \mathbf{q} on $[-r, 0]$.

For example, consider a scalar delay differential equation

$$\dot{x}(t) = a_0(t)x(t) + a_1(t)x(t-1) + a_2(t)x(t-3). \quad (4.25)$$

Define:

$$\mathbf{h}(t, \mathbf{q}) = \begin{cases} 0 & \mathbf{q} \geq 0 \\ -a_0(t) & -1 \leq \mathbf{q} \leq 0 \\ -a_0(t) - a_1(t) & -3 \leq \mathbf{q} \leq -1 \\ -a_0(t) - a_1(t) - a_2(t) & \mathbf{q} \leq -3 \end{cases} \quad (4.26)$$

$$= -a_0(t) \mathbf{c}_{(-\infty, 0)}(\mathbf{q}) - a_1(t) \mathbf{c}_{(-\infty, -1)}(\mathbf{q}) - a_2(t) \mathbf{c}_{(-\infty, -3)}(\mathbf{q}) \quad (4.27)$$

where $\mathbf{c}_I(\cdot)$ is the characteristic function defined on the interval I . Then, equation (4.25) can be written as

$$\dot{x}(t) = \int_{-3}^0 [d_{\mathbf{q}} \mathbf{h}(t, \mathbf{q})] x(t + \mathbf{q}) \quad (4.28)$$

where for fixed t , the function $\mathbf{q} \rightarrow \mathbf{h}(t, \mathbf{q})$ is piecewise constant.

In the case of linear delay differential equations, a connection with the theory of partial differential equations has been established. More specifically, in earlier papers the solution in the space C to the linear autonomous equation

$$\dot{x}(t) = L(x_t), \quad (4.29)$$

where

$$L(\mathbf{f}) = \int_{-r}^0 [d\mathbf{h}(\mathbf{q})] \mathbf{f}(\mathbf{q}), \quad (4.30)$$

can be written as $x_t = T(t)x_0$, where x_t denotes a segment of the trajectory $s \rightarrow x(s)$, $t - r \leq s \leq t$ and $T(t)$ is a solution operator $T: C \rightarrow C$ [61]. It has been shown that the family $\{T(t), t \geq 0\}$ is a semigroup of linear transformations, that $t \rightarrow T(t)$ is strongly continuous on $[0, \infty)$, and $T(t): C \rightarrow C$ is compact for $t \geq s$. The infinitesimal generator A of $T(t)$ is characterized by

$$D(A) = \{ \mathbf{f} \in C \mid \dot{\mathbf{f}} \in C, \dot{\mathbf{f}}(0) = L(\mathbf{f}) \} \quad (4.31)$$

$$(A\mathbf{f})(\mathbf{q}) = \begin{cases} L(\mathbf{f}) & \mathbf{q} = 0 \\ \frac{d\mathbf{f}(\mathbf{q})}{d\mathbf{q}} & -r \leq \mathbf{q} \leq 0 \end{cases} \quad (4.32)$$

Subsequently, some other spaces have also been introduced including embedding the system states into a larger space, which is the space of pairs $\mathbf{f} = (\mathbf{f}^0, \mathbf{f}^1)$, $\mathbf{f}^0 \in \mathbb{R}^n$, $\mathbf{f}^1 \in L_2([-r, 0], \mathbb{R}^n)$ with the norm

$$\|\mathbf{f}\|_{\mathbb{R}^n \times L_2} = \left(\|\mathbf{f}^0\|_{\mathbb{R}^n}^2 + \int_{-r}^0 \|\mathbf{f}^1(\mathbf{q})\|_{\mathbb{R}^n}^2 d\mathbf{q} \right)^{\frac{1}{2}}. \quad (4.33)$$

In [62] system equations of the following form have been considered:

$$\begin{aligned} \dot{x}(t) = & A_0(t)x(t) + \sum_{i=1}^N A_i(t)x(t-h_i) \\ & + \int_{-h}^0 A_{01}(t, \mathbf{q})x(t+\mathbf{q})d\mathbf{q} \quad t \in [t_0, t_1] \end{aligned} \quad (4.34)$$

$$x(t) = \begin{cases} \mathbf{f}^0 & t = t_0 \\ \mathbf{f}^1(t-t_0) & t_0 - h \leq t \leq t_0 \end{cases} \quad (4.35)$$

where $0 = h_0 \leq h_1 \dots \leq h_N \leq h$, $r = h$, the matrix functions $A_0(\cdot)$, $A_i(\cdot)$ are bounded measurable on $[t_0, t_1]$ and $A_{01}(\cdot, \cdot)$ is bounded measurable on $[t_0, t_1] \times [-h, 0]$. Note that continuity of the initial function is not required, as \mathbf{f}^0 is treated as an element independent of \mathbf{f}^1 .

Under those assumptions it has been proven [62] that the solution $t \rightarrow x(t)$ of equation (4.34) with initial condition (4.35) exists and is unique, and is a continuous

function of t , $t \in [t_0, t_1]$. Corresponding to this solution, there is an element

$\tilde{x}(t) \in M_2 = \mathbb{R}^n \times L_2([-h, 0], \mathbb{R}^n)$ given by

$$\tilde{x}(t) = (\tilde{x}(t)^0, \tilde{x}(t)^1) \quad (4.36)$$

$$\tilde{x}(t)^0 = x(t) \quad (4.37)$$

$$\tilde{x}(t)^1(\mathbf{q}) = x(t + \mathbf{q}), \quad \mathbf{q} \in [-h, 0]. \quad (4.38)$$

Define W^2 as a subspace of M_2 given by

$$W^2 = \{(\mathbf{f}(0), \mathbf{f}) \in M_2 \mid \mathbf{f} \in W_2^{(1)}([-h, 0], \mathbb{R}^n)\} \quad (4.39)$$

where $W_2^{(1)}([-h, 0], \mathbb{R}^n)$ is the space of absolutely continuous functions from $[-h, 0]$ to \mathbb{R}^n with first derivative in $L_2([-h, 0], \mathbb{R}^n)$. It has been shown [62] that if the initial function $\mathbf{f} \in W_2^{(1)}$, then $\tilde{x}(t) \in W^2$ for all $t \geq t_0$, and $t \rightarrow \tilde{x}(t)$ is strongly continuous in the space W^2 endowed with the norm topology of $W_2^{(1)}$.

Therefore, one can transform (4.34) into an abstract form

$$\begin{aligned} \frac{d\tilde{x}(t)}{dt} &= \tilde{A}(t)\tilde{x}(t) \\ \tilde{x}(t_0) &= \mathbf{f} \end{aligned} \quad (4.40)$$

where $\tilde{A}(t)$ is a linear operator defined by

$$[\tilde{A}(t)\mathbf{f}]^0 = A_0(t)\mathbf{f}(0) + \sum_{i=1}^N A_i(t)\mathbf{f}(-h_i) + \int_{-r}^0 A_{01}(t, \mathbf{q})\mathbf{f}(\mathbf{q})d\mathbf{q} \quad (4.41)$$

$$[\tilde{A}(t)\mathbf{f}]^1(\mathbf{q}) = \frac{d\mathbf{f}}{d\mathbf{q}}(\mathbf{q}), \quad \mathbf{q} \in (-h, 0). \quad (4.42)$$

Furthermore, equations (4.41)-(4.42) and (4.35) can be seen to represent a mixed initial-boundary value problem for hyperbolic partial differential equations. That is, let $z(t, \mathbf{q})$ be defined as

$$z(t, \mathbf{q}) = x(t + \mathbf{q}) \quad (4.43)$$

then $z(t, \mathbf{q})$ can be seen to be a solution to

$$\frac{\partial z(t, \mathbf{q})}{\partial t} = \frac{\partial z(t, \mathbf{q})}{\partial \mathbf{q}} \quad \text{in} \quad (t_0, t_1) \times (-h, 0) \quad (4.44)$$

with boundary condition

$$\frac{\partial}{\partial t} z(t, 0) = A_0(t)z(t, 0) + \sum_{i=1}^N A_i(t)z(t, -h_i) + \int_{-h}^0 A_{01}(t, \mathbf{q})z(t, \mathbf{q}) d\mathbf{q} \quad (4.45)$$

in (t_0, t_1) and initial condition

$$z(0, \mathbf{q}) = \mathbf{f}(\mathbf{q}) \quad \mathbf{q} \in (-h, 0). \quad (4.46)$$

Similar representations of linear retarded functional differential equations were given in many other reports since they are by now well-known to investigators working on these problems. In [63] an approximation scheme was formulated and convergence in the context of known results from linear semigroup theory was given.

4.3 Review on Lyapunov Stability Theory for Linear Systems

In this section, we shall restrict ourselves to linear autonomous systems, i.e., systems described by

$$\dot{x}(t) = Ax(t) \quad (4.47)$$

In this special case, Lyapunov theory is very complete. In particular, we have the following theorem which is well-known (see e.g. [64]).

Theorem 1: The equilibrium point 0 of (4.47) is (globally) asymptotically stable if and only if all eigenvalues of A have negative real parts. The equilibrium point 0 of (4.47) is stable if and only if all eigenvalues of A have zero real parts and every eigenvalue of A having a zero real part is a simple zero of the minimal polynomial of A .

Thus, in the case of linear time-invariant systems of the form (4.47) the stability of the equilibrium point 0 can be determined by studying the eigenvalues of A . However, an alternative approach can be formulated for the stability analysis by using quadratic Lyapunov functions. This theory is of interest in itself and will be extended in the next section for the linear function differential equation.

Sometimes referred to as “energy functions”, Lyapunov functions have the property that it is zero at the origin and positive everywhere else. In other words, the energy of the system has its global minimum at 0 . Now, suppose the system, which is originally at the equilibrium state 0 , is perturbed into a nonzero initial state. If the system dynamics are such that the energy of the system is nonincreasing with time, then the energy of the system never increases beyond its initial positive value. Depending on the nature of the energy function, this may be enough to imply the stability of the equilibrium point 0 . On the other hand, if the system dynamics are such that the energy of the system is monotonically decreasing with time and the energy eventually reduces to zero, then under suitable additional assumptions it may be possible to conclude the asymptotic stability of the equilibrium state 0 .

More precisely, we have the following well-known results [64]:

Theorem 2: The equilibrium point 0 at time t_0 of (4.47) is stable if there exists a continuously differentiable locally positive definite function V such that

$$\dot{V}(t, x) \leq 0, \quad \forall t \geq t_0, \quad \forall x \in B_r = \{x \mid \|x\| \leq r\}, \quad (4.48)$$

$r > 0$.

Theorem 3: The equilibrium point 0 at time t_0 of (4.47) is (uniformly) asymptotically stable over the interval $[t_0, \infty)$ if there exists a continuously differentiable locally positive definite function V such that $-\dot{V}$ is a locally positive definite function.

For linear system (4.47), a Lyapunov function candidate has the form

$$V(x) = x^T P x \quad (4.49)$$

where P is a real symmetric matrix. Then \dot{V} is given by

$$\begin{aligned} \dot{V}(x) &= \dot{x}^T P x + x^T P \dot{x} \\ &= x^T [A^T P + P A] x \\ &= -x^T Q x \end{aligned} \quad (4.50)$$

where

$$A^T P + P A = -Q. \quad (4.51)$$

Equation (4.49) is known as the Lyapunov matrix equation. By means of this equation, one can study the stability properties of the equilibrium point 0. For example, if a pair of matrices (P, Q) satisfying (4.51) can be found so that both P and Q are positive definite, then both V and $-\dot{V}$ are positive definite functions. Hence by Theorem 3, the equilibrium point 0 of (4.47) is (globally) asymptotically stable.

We now state one of the main results for the Lyapunov matrix equation that can be used to enable one to unambiguously determine whether or not 0 is an asymptotically stable equilibrium point.

Theorem 4: Given a matrix $A \in \mathbb{R}^{n \times n}$, the following three statements are equivalent:

- (i.) All eigenvalues of A have negative real parts.
- (ii.) There exists some positive definite matrix $Q \in \mathbb{R}^{n \times n}$ such that (4.51) has a unique positive definite solution P .
- (iii.) For every positive definite matrix $Q \in \mathbb{R}^{n \times n}$, (4.51) has a unique positive definite solution P .

In practice, one applies Theorem 4 in the following manner: Given $A \in \mathbb{R}^{n \times n}$, pick $Q \in \mathbb{R}^{n \times n}$ to be any positive definite matrix (e.g., $Q = I$) and solve (4.51) for P . If (4.51) has no solution or if it has more than one solution, then 0 is not asymptotically stable. Suppose that (4.51) does have a unique solution but this solution is not positive definite, then again 0 is not asymptotically stable. On the other hand, if this unique solution is also positive definite, then 0 is a globally asymptotically stable equilibrium point of (4.47).

These ideas will be extended in the next section to the linear differential-difference equations with multiple delays.

4.4 Stability Conditions for Delay Differential Equations with Multiple Time Delays

Several authors [37-40, 42-47, 65-73] have studied the stability of linear systems with time delays. Two approaches to study the stability of delay differential equations is through the use of matrix norms (e.g. [43, 44]) and linear matrix inequalities, or LMI's (e.g. [43, 44, 74]). While these methods are reliable, calculations can quickly become tedious. In a similar manner, results that attempt to state stability conditions for delay differential equations by adapting the direct Lyapunov method face the problem of highly complex mathematical formulations. For example, Kolmanovskii and Richard [75] consider the following simple delay differential equation:

$$\dot{x}(t) = Ax(t) + Bx(t-h) \quad (4.52)$$

where $h \geq 0$ is a constant delay and $A, B \in \mathbb{R}^{n \times n}$ are constant matrices. Following the Lyapunov theory for linear systems without delay presented in the previous section, a Lyapunov function candidate for (4.52) has a quadratic form

$$\begin{aligned} V = & x^T(t)Px(t) + x^T(t) \int_{-h}^0 P_1(s)x(t+s)ds \\ & + x(t) \int_{-h}^0 x^T(t+s)P_1^T(s)ds \\ & + \int_{-h}^0 \int_{-h}^0 x^T(t+s)P_2(s, \mathbf{t})x(t+\mathbf{t})dsd\mathbf{t}. \end{aligned} \quad (4.53)$$

The negativity of the derivative of V along the solution of system (4.52) implies that the matrices P, P_1, P_2 must satisfy a complex system of algebraic, ordinary and even partial differential equations with the appropriate boundary conditions. Some authors [41, 69, 71, 72] circumvent this obstacle by using concrete functionals V for special classes of

delays equations; thus obtaining stability conditions directly in terms of the system's coefficients.

However, Kolmanovskii and Richard note that the results reported by various authors [66, 67, 69, 72, 74, 76, 77] show that the form of Riccati equation depends on a particular choice of Lyapunov functional, V . Thus, they present the argument that if the same equation has various functionals V , different Riccati equations are attainable and as a result, the estimate of the stability domain in the space of parameters can be expanded. Their paper applies a formal procedure (discussed and used in [71, 78, 79]) to construct a functional V for concrete delay equations. The authors obtain delay-dependent and delay-independent stability conditions formulated in terms of the existence of positive definite solutions of Riccati equations for systems that have the general form

$$\dot{x}(t) = \sum_{i=1}^m B_i x(t - h_i) + \sum_{j=1}^r C_j \int_{t-t_j}^t x(s) ds \quad (4.54)$$

where $t \geq 0$, B_i, C_j are constant matrices in $\mathbb{R}^{n \times n}$, $h_i \geq 0$ and $t_j \geq 0$ are constant. The initial conditions are on the interval $[-h_0, 0]$, with $h_0 = \max_{i,j} (h_i, t_j)$. In the next sequel, delay-dependent stability conditions are derived for delay differential equations with multiple discrete delays. The reformulation of the first moments for the spermatocytogenesis CGD model (see Section 4.1) allows the system's stability properties to be studied using the theory outlined below.

Consider the following discrete delay system:

$$\dot{x}(t) = \sum_{i=1}^m B_i x(t - h_i). \quad (4.55)$$

Note that equation (4.55) is equivalent to the following neutral functional differential equation:

$$\frac{d}{dt} \left[x(t) + \sum_{i=1}^m B_i \int_{t-h_i}^t x(s) ds \right] = Ax(t) \quad (4.56)$$

where $A = \sum_{i=1}^m B_i$.

Theorem 5: Assume that for some symmetric matrices $R_i > 0$ and $Q > 0$ there exists a solution, $P > 0$, of the Riccati equation

$$A^T P + PA + \sum_{i=1}^m R_i h_i + \sum_{i=1}^m A^T P B_i R_i^{-1} B_i^T P A h_i = -Q \quad (4.57)$$

where $A = \sum_{i=1}^m B_i$. Then equation (4.55) is asymptotically.

Proof: We start by choosing a Lyapunov function candidate $V = V_1 + V_2$ where

$$V_1 = \left[x(t) + \sum_{i=1}^m B_i \int_{t-h_i}^t x(s) ds \right]^T P \left[x(t) + \sum_{i=1}^m B_i \int_{t-h_i}^t x(s) ds \right] \quad (4.58)$$

and

$$V_2 = \sum_{i=1}^m \int_0^{h_i} ds \int_{t-s}^t x^T(\mathbf{t}) R_i x(\mathbf{t}) d\mathbf{t}. \quad (4.59)$$

Observe that

$$\begin{aligned} \dot{V}_1 = & \left[\dot{x}(t) + \sum_{i=1}^m B_i x(t) - \sum_{i=1}^m B_i x(t-h_i) \right]^T P \left[x(t) + \sum_{i=1}^m B_i \int_{t-h_i}^t x(s) ds \right] \\ & + \left[x(t) + \sum_{i=1}^m B_i \int_{t-h_i}^t x(s) ds \right]^T P \left[\dot{x}(t) + \sum_{i=1}^m B_i x(t) - \sum_{i=1}^m B_i x(t-h_i) \right] \end{aligned} \quad (4.60)$$

$$\begin{aligned} = & \left[\sum_{i=1}^m B_i x(t-h_i) + \sum_{i=1}^m B_i x(t) - \sum_{i=1}^m B_i x(t-h_i) \right]^T P \left[x(t) + \sum_{i=1}^m B_i \int_{t-h_i}^t x(s) ds \right] \\ & + \left[x(t) + \sum_{i=1}^m B_i \int_{t-h_i}^t x(s) ds \right]^T P \left[\sum_{i=1}^m B_i x(t-h_i) + \sum_{i=1}^m B_i x(t) - \sum_{i=1}^m B_i x(t-h_i) \right] \end{aligned} \quad (4.61)$$

$$\begin{aligned} = & x(t)^T A^T P x(t) + x(t)^T A^T P \sum_{i=1}^m B_i \int_{t-h_i}^t x(s) ds + x(t)^T P A x(t) \\ & + \int_{t-h_i}^t x^T(s) ds \sum_{i=1}^m B_i^T P A x(t). \end{aligned} \quad (4.62)$$

Also note that

$$\dot{V}_2 = \sum_{i=1}^m \int_0^{h_i} \frac{d}{dt} \left[\int_{t-s}^t x^T(t) R_i x(t) dt \right] ds \quad (4.63)$$

$$= \sum_{i=1}^m \int_0^{h_i} \left[x^T(t) R_i x(t) - x^T(t-s) R_i x(t-s) \right] ds \quad (4.64)$$

$$= \sum_{i=1}^m \left[h_i x^T(t) R_i x(t) - \int_0^{h_i} x^T(t-s) R_i x(t-s) ds \right] \quad (4.65)$$

$$= \sum_{i=1}^m h_i x^T(t) R_i x(t) - \sum_{i=1}^m \int_{t-h_i}^t x^T(s) R_i x(s) ds. \quad (4.66)$$

Thus,

$$\begin{aligned} \dot{V} = & x(t)^T \left[A^T P + P A + \sum_{i=1}^m h_i R_i \right] x(t) \\ & + \sum_{i=1}^m \int_{t-h_i}^t \left[x(t)^T A^T P B_i x(s) + x^T(s) B_i^T P A x(t) - x^T(s) R_i x(s) \right] ds. \end{aligned} \quad (4.67)$$

$$\begin{aligned}
&= x(t)^T \left[A^T P + PA + \sum_{i=1}^m h_i R_i + \sum_{i=1}^m A^T P B_i R_i^{-1} B_i^T P A h_i \right] x(t) \\
&\quad - \sum_{i=1}^m \int_{t-h_i}^t \left[x(t)^T A^T P B_i R_i^{-1} B_i^T P A + x^T(t) A^T P B_i B_i^T x(s) \right. \\
&\quad \quad \left. + x^T(s) B^T P A x(t) - x^T(s) R_i x(s) \right] ds
\end{aligned} \tag{4.68}$$

$$\begin{aligned}
&= x(t)^T \left[A^T P + PA + \sum_{i=1}^m h_i R_i + \sum_{i=1}^m A^T P B_i R_i^{-1} B_i^T P A h_i \right] x(t) \\
&\quad - \sum_{i=1}^m \int_{t-h_i}^t \left[x(t)^T A^T P B_i R_i^{-1} - x^T(s) R_i R_i^{-1} \right] \left[B_i^T P A x(t) - R_i x(s) \right] ds
\end{aligned} \tag{4.69}$$

$$\begin{aligned}
&= x(t)^T \left[A^T P + PA + \sum_{i=1}^m h_i R_i + \sum_{i=1}^m A^T P B_i R_i^{-1} B_i^T P A h_i \right] x(t) \\
&\quad - \sum_{i=1}^m \int_{t-h_i}^t \left[x(t)^T A^T P B_i - x^T(s) R_i \right] R_i^{-1} \left[B_i^T P A x(t) - R_i x(s) \right] ds
\end{aligned} \tag{4.70}$$

$$\begin{aligned}
&= x(t)^T \left[A^T P + PA + \sum_{i=1}^m h_i R_i + \sum_{i=1}^m A^T P B_i R_i^{-1} B_i^T P A h_i \right] x(t) \\
&\quad - \sum_{i=1}^m \int_{t-h_i}^t \left[x(t)^T A^T P B_i - x^T(s) R_i \right] R_i^{-1} \left[x(t)^T A^T P B_i - x^T(s) R_i \right]^T ds
\end{aligned} \tag{4.71}$$

Under the assumptions of Theorem 5, \dot{V} is negative and equation (4.56) is asymptotically stable. q.e.d.

We note that the Lyapunov function given in the proof of Theorem 5 for the linear system (4.56) is not unique. For example, equation (4.55) can also be written as the following functional differential equation:

$$\dot{x}(t) = Ax(t) - \sum_{i=1}^m B_i \int_{t-h_i}^t \dot{x}(s) ds. \tag{4.72}$$

Theorem 6: Assume that for some symmetric matrices $R > 0$ and $Q > 0$ there exists a solution, $P > 0$, of the Riccati equation

$$A^T P + PA + \sum_{i=1}^m \left(h_i P B_i R^{-1} B_i^T P + m h B_i^T R B_i \right) = -Q \quad (4.73)$$

where $A = \sum_{i=1}^m B_i$ and $h = \sum_{i=1}^m h_i$. Then equation (4.55) is asymptotically stable.

Proof: We chose the Lyapunov function candidate $V = V_1 + V_2 + V_3$ where

$$V_1 = x^T(t) P x(t) \quad (4.74)$$

$$V_2 = \sum_{i=1}^m \int_0^{h_i} ds \int_{t-s}^t \dot{x}(t) R_i \dot{x}(t) dt \quad (4.75)$$

and

$$V_3 = m h \sum_{i=1}^m \int_{t-h_i}^t x^T(s) B_i^T R B_i x(s) ds. \quad (4.76)$$

Observe that

$$\dot{V}_1 = \dot{x}^T(t) P x(t) + x(t) P \dot{x}(t) \quad (4.77)$$

$$= \left[Ax(t) - \sum_{i=1}^m B_i \int_{t-h_i}^t \dot{x}(s) ds \right]^T P x(t) + x^T(t) P \left[Ax(t) - \sum_{i=1}^m B_i \int_{t-h_i}^t \dot{x}(s) ds \right] \quad (4.78)$$

$$= x(t)^T [A^T P + PA] x(t) - \sum_{i=1}^m \int_{t-h_i}^t \left[x^T(s) B_i^T P x(t) + x^T(t) P B_i \dot{x}(s) \right] ds. \quad (4.79)$$

Note that

$$\dot{V}_2 = \sum_{i=1}^m \int_0^{h_i} \frac{d}{dt} \left[\int_{t-s}^t \dot{x}^T(t) R \dot{x}(t) dt \right] ds \quad (4.80)$$

$$= \sum_{i=1}^m \int_0^{h_i} \left[\dot{x}^T(t) R \dot{x}(t) - \dot{x}^T(t-s) R \dot{x}(t-s) \right] ds \quad (4.81)$$

$$= \sum_{i=1}^m h_i \dot{x}^T(t) R \dot{x}(t) - \sum_{i=1}^m \int_0^{h_i} \dot{x}^T(t-s) R \dot{x}(t-s) ds \quad (4.82)$$

$$= \sum_{i=1}^m h_i \dot{x}^T(t) R \dot{x}(t) - \sum_{i=1}^m \int_{t-h_i}^t \dot{x}^T(s) R \dot{x}(s) ds. \quad (4.83)$$

Similarly,

$$\dot{V}_3 = mh \sum_{i=1}^m \frac{d}{dt} \left[\int_{t-h_i}^t x^T(s) B_i^T R B_i x(s) ds \right] \quad (4.84)$$

$$= mh \sum_{i=1}^m \left[x^T(t) B_i^T R B_i x(t) - x^T(t-h_i) B_i^T R B_i x(t-h_i) \right]. \quad (4.85)$$

Thus,

$$\dot{V} = \dot{V}_1 + \dot{V}_2 + \dot{V}_3 \quad (4.86)$$

$$\begin{aligned} &= x(t)^T \left[A^T P + P A \right] x(t) \\ &\quad - \sum_{i=1}^m \int_{t-h_i}^t \left[\dot{x}^T(s) B_i^T P x(t) + x^T(t) P B_i \dot{x}(s) + \dot{x}^T(s) R \dot{x}(s) \right] ds \\ &\quad + \sum_{i=1}^m h_i \dot{x}^T(t) R x(t) + \dot{V}_3. \end{aligned} \quad (4.87)$$

To further simplify the expression for \dot{V} , note that the integrand of the second term in equation (4.87) can be written in the following manner:

$$-\dot{x}^T(s) B_i^T P x(t) - x^T(t) P B_i \dot{x}(s) - \dot{x}^T(s) R \dot{x}(s) \quad (4.88)$$

$$\begin{aligned} &= -[\dot{x}^T(s) B_i^T P x(t) + x^T(t) P B_i R^{-1} R \dot{x}(s) + \dot{x}^T(s) R \dot{x}(s) \\ &\quad + x^T(t) P B_i R^{-1} B_i^T P x(t) - x^T(t) P B_i R^{-1} B_i^T P x(t)] \end{aligned} \quad (4.89)$$

$$= -[\dot{x}^T(s) R R^{-1} + x^T(t) P B_i R^{-1}] [B_i^T P x(t) + R \dot{x}(s)] + x^T(t) P B_i R^{-1} B_i^T P x(t) \quad (4.90)$$

$$= -[B_i^T P x(t) + R \dot{x}(s)]^T R^{-1} [B_i^T P x(t) + R \dot{x}(s)] + x^T(t) P B_i R^{-1} B_i^T P x(t). \quad (4.91)$$

In equation (4.87) the last two terms can be combined as

$$\begin{aligned}
h\dot{x}^T(t)R\dot{x}(t) + \dot{V}_3 &= h \left[\sum_{i=1}^m B_i x(t-h_i) \right]^T R \left[\sum_{j=1}^m B_j x(t-h_j) \right] \\
&+ mh \sum_{i=1}^m \left[x^T(t) B_i^T R B_i x(t) - x^T(t-h_i) B_i^T R B_i x(t-h_i) \right]
\end{aligned} \tag{4.92}$$

$$\begin{aligned}
&= h \sum_{i=1}^m x^T(t-h_i) B_i^T R \sum_{j=1}^m B_j x(t-h_j) \\
&+ mh \sum_{i=1}^m \left[x^T(t) B_i^T R B_i x(t) - x^T(t-h_i) B_i^T R B_i x(t-h_i) \right]
\end{aligned} \tag{4.93}$$

$$\begin{aligned}
&= h \sum_{i,j=1}^m x^T(t-h_i) B_i^T R B_j x(t-h_j) \\
&+ mh \sum_{i=1}^m x^T(t) B_i^T R B_i x(t) - mh \sum_{i=1}^m x^T(t-h_i) B_i^T R B_i x(t-h_i).
\end{aligned} \tag{4.94}$$

But note that from the first and last terms of equation (4.94) we have the following

$$\begin{aligned}
&\sum_{i,j=1}^m x^T(t-h_i) B_i^T R B_j x(t-h_j) - m \sum_{i=1}^m x^T(t-h_i) B_i^T R B_i x(t-h_i) \\
&= -(m-1) \sum_{i=1}^m x^T(t-h_i) B_i^T R B_i x(t-h_i) + \sum_{\substack{i,j=1 \\ i \neq j}}^m x^T(t-h_i) B_i^T R B_j x(t-h_j) \\
&= - \left[\sum_{i=1}^m x^T(t-h_i) B_i^T R B_i x(t-h_i) (m-i) - \sum_{i=2}^m (1-i) x^T(t-h_i) B_i^T R B_i x(t-h_i) \right] \\
&\quad + \sum_{\substack{i,j=1 \\ i \neq j}}^m x^T(t-h_i) B_i^T R B_j x(t-h_j)
\end{aligned} \tag{4.95}$$

Here, we note that $\sum_{i=2}^m (1-i) x^T(t-h_i) B_i^T R B_i x(t-h_i) = \sum_{i=1}^m (1-i) x^T(t-h_i) B_i^T R B_i x(t-h_i)$,

so that equation (4.96)

$$= - \left[\sum_{i=1}^m x^T(t-h_i) B_i^T R B_i x(t-h_i) \sum_{j=i+1}^m 1 - \sum_{i=1}^m \sum_{j=i+1}^m x^T(t-h_j) B_j^T R B_j x(t-h_j) \right] + \sum_{\substack{i,j=1 \\ i \neq j}}^m x^T(t-h_i) B_i^T R B_j x(t-h_j) \quad (4.97)$$

$$= - \sum_{\substack{i=1 \\ j=i+1}}^m \left[x^T(t-h_i) B_i^T R B_i x(t-h_i) + x^T(t-h_j) B_j^T R B_j x(t-h_j) \right] + \sum_{\substack{i,j=1 \\ i \neq j}}^m x^T(t-h_i) B_i^T R B_j x(t-h_j) \quad (4.98)$$

$$= - \sum_{\substack{i=1 \\ j=i+1}}^m \left[x^T(t-h_i) B_i^T R B_i x(t-h_i) - x^T(t-h_i) B_i^T R B_j x(t-h_j) - x^T(t-h_j) B_j^T R B_i x(t-h_i) + x^T(t-h_j) B_j^T R B_j x(t-h_j) \right] \quad (4.99)$$

$$= - \sum_{\substack{i=1, \\ j=i+1}}^m \left[B_i x(t-h_i) - B_j x(t-h_j) \right]^T R \left[B_i x(t-h_i) - B_j x(t-h_j) \right]. \quad (4.100)$$

Observe that in equation (4.100) for $i = m$ the summand equals zero. Thus, we get

$$\begin{aligned} \dot{V} &= x(t)^T \left[A^T P + PA + \sum_{i=1}^m (h_i P B_i R^{-1} B_i^T P + m h B_i^T R B_i) \right] x(t) \\ &\quad - \sum_{i=1}^m \int_{t-h_i}^t \left[B_i^T P x(t) + R \dot{x}(s) \right]^T R^{-1} \left[B_i^T P x(t) + R \dot{x}(s) \right] ds \\ &\quad - h \sum_{\substack{i=1, \\ j=i+1}}^m \left[B_i x(t-h_i) - B_j x(t-h_j) \right]^T R \left[B_i x(t-h_i) - B_j x(t-h_j) \right]. \end{aligned} \quad (4.101)$$

The derivative of V is negative under the assumptions of Theorem 6. q.e.d.

4.5 Stability Analysis of Spermatocytogenesis CGD Model

The first approach taken to study the stability of the spermatocytogenesis CGD model was to apply one of the theorems developed in Section 4.4. For a system of dimension two, Kolmanovskii and Richard were able to explicitly express a relationship between the delays and the coefficient matrices for a delay-dependent stability condition. In a similar example, they also explicitly expressed a delay-independent stability condition. However, simply increasing the dimension of the system to three adds a great level of difficulty and an explicit expression for stability may not be attainable ([37, 38]). This was also the case with the spermatocytogenesis CGD model. As an alternative, we investigated the stability of the system for specific parameter values (see Table 5 and Table 6). Using the time delays and the coefficient matrices for the spermatocytogenesis CGD model (see Section 4.1) the Riccati matrix equation was set up based on Theorem 6:

$$A^T P + PA + \sum_{i=1}^m \left(h_i P B_i R^{-1} B_i^T P + m h B_i^T R B_i \right) = -Q \quad (4.102)$$

where $m = 7$, $Q = I_{7 \times 7}$ and $R_i = I_{7 \times 7}$ for $i = 1, \dots, 7$.

The optimization toolbox in MatLab uses an algorithm designed to find a unique matrix solution for the Riccati equation (i.e., a symmetric positive definite matrix P). If a matrix solution exists, the algorithm returns the stabilizing solution to the Riccati equation defined in (4.102). The parameter values listed in Table 6 yield a symmetric positive definite matrix P for the above Riccati equation. Thus, the spermatocytogenesis CGD model using the developmental rates listed in Table 6 is

asymptotically stable. However, the parameter values listed in Table 5 did not yield a symmetric positive matrix P for the Riccati equation defined in (4.102). This simply means that the results for the system's stability using these particular parameters are inconclusive.

Chapter 5: Discussion and Future Directions

This chapter encapsulates the research that has been presented in this dissertation. Section 5.1 is a discussion of the proposed CGD model. Possible future direction and research of this investigation is reviewed in Section 5.2. Finally, Section 5.3 presents a brief summary of the research performed.

5.1 Discussion

A controlled growth and differentiation (CGD) model was developed by extending a model originally developed by Leroux et al. The CGD model was designed to allow the modeler to include an indefinite number of intermediate stages of development. These intermediate stages are intended to make the system of differential equations better mimic the biological process under study. The CGD model also provides information about the second moments of the random variables. To demonstrate the power of the CGD model, a model of normal germ cell development in the male body was designed. The spermatogenesis model included time delays to

replicate the actual time of development of germ cells in the male testes. Since no experimental data at various stages of cell development was available (to the best of the author's knowledge), two parameter sets were used with the model to investigate the behavior of the system. Although the first parameter set demonstrated chaotic behavior initially, as the independent variable of time approached infinity the system reached a state of equilibrium. The second parameter set caused the system to behave in a less chaotic manner and produced curves that more closely resemble true sperm cell production. The ability of the CGD model to mimic a biological process that resembles a hormetic effect was also investigated. The developmental rates in the CGD model were altered to yield a dose-response relationship. The CGD spermatocytogenesis model produced a beta curve that signifies a toxic agent having a hormetic effect on cell production. The ability of the CGD model to ultimately predict a stable population of committed cells is a key feature not found in the Leroux et al. model. This feature moves biological modeling closer to being able to mimic reality.

After the CGD spermatocytogenesis model with time lags was established, the system was reformulated to allow an examination of existence and uniqueness properties. The equivalent system was used to study delay-dependent stability conditions. Based on the theory for delay differential equations, the CGD spermatogenesis model with multiple discrete time delays is asymptotically stable under the assumptions presented in the theorems in Section 4.4. However, due to the dimensions of the system, a closed form solution could not readily be identified. An attempt was made to verify the theoretical results on stability by numerical simulations.

This was accomplished with one parameter set, but the other parameter set yielded inconclusive information.

5.2 Directions for Future Research

The development of the CGD model has contributed to the advancement of biologically based mathematical models. The applications presented in this dissertation only scratch the surface of all the possible models that can be designed using the framework of the CGD model. For example, the CGD model can be adapted to model the probability of cancer incidence. A simple model would include normal, initiated and malignant cell populations. These basic states could be augmented to further replicate the possible pathway a cell follows to ultimately become part of a malignant focal lesion.

The CGD model may also be used as an added component of physiologically based pharmacokinetic (PBPK) modeling. PBPK models quantify the absorption, metabolism and excretion of chemicals. These models also use dose-dependent developmental rates, just as the CGD model. The resulting information from PBPK models include the concentration amount of a chemical, i.e., the dose amount present in the system. Thus, the PBPK model and the CGD model can be combined to produce quantitative information on the concentration level of a chemical in a given system and the effect it produces on a developing tissue or organ.

The method used to derive the CGD model may also be applied to the development of PBPK models. Thus, PBPK models can be made stochastic thereby increasing its quantitative capabilities.

5.3 Summary

This dissertation extended and studied the mechanisms of a biologically based mathematical model that can be used to predict a host of biological phenomena. The CGD model was inspired by a model originally proposed by Leroux, Leisenring, Moolgavkar and Fautsman. The original model included precursor cells and differentiated cells. This limitation unrealistically forced all biological systems to have cells in one of the two subpopulations. However, the CGD model was designed to allow for various intermediate stages of development. The idea that a cell passes through a maturation process before transforming to a differentiated state is a key feature that was developed in the CGD model. Thus, the model is able to limit the number of replications allowed in the development of a tissue or organ. A comparison of the Leroux et al. model and the CGD model revealed that the CGD model is highly versatile and thus a more powerful tool in the area of biological modeling.

In particular, the CGD model was used to explore the biological process of normal sperm development. The CGD spermatocytogenesis model was then investigated to test its versatility. This dissertation also explored the adaptability of the CGD model to biological development as it relates to toxic agents with hormetic effects.

Time lags were included in the CGD spermatocytogenesis model in an effort to create a more biologically realistic model. Existence and uniqueness of the delay differential equations were studied by reformulating the original spermatocytogenesis CGD model. Stability conditions were examined for delay differential equations with multiple discrete time delays. The theoretical stability study conducted with the spermatocytogenesis CGD model indicated that stability criteria exists for the system of delay differential equations; however, due to the size of the system, these criteria could not be explicitly expressed in closed form.

As a result of the topics investigated in this dissertation, more complicated models can be used to study various biological processes. The method used to derive the CGD model also introduces stochasticity into biological modeling. As a result, the CGD model is an enhancement to the class of models used to study cellular growth kinetics, hormesis, physiologically based pharmacokinetics and cancer incidence.

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