SOME STOCHASTIC MODELS FOR MICROORGANISM DEATH KINETICS

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This is to certify that the

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ABSTRACT

SOME STOCHASTIC MODELS FOR MICROORGANISM DEATH KINETICS

by Frederick Pierce Geyer

Mathematical models currently used for microbial death kinetics are deterministic. Little attention has been given to population fluctuations arising from random aspects inherent in death processes. Modern probability theory was used to show how quantitative values can be assigned to the influence of these factors on the population size during a reduction process. Consequently, real death processes were not studied, but instead a method of mathematical modeling was derived and illustrated.

Death processes were considered as Markov processes with a continuous time parameter. Chapman-Kolmogorov equations were derived and solved by the use of probability generating functions. Models for organisms with one and two viable states were considered for both time dependent and constant death rates.

The stochastic models obtained gave theoretical probability distributions for the discrete levels of possible population size during a reduction process. The mean of the probability distribution derived was equivalent to the prediction of deterministic models. The latter was

found to give a good approximation of the stochastic model for determining the lethal treatment required to sterilize a population of microorganisms.

From the theoretical probability distributions derived, several methods were given to simulate a population reduction process. These techniques were computer programmed to generate simulated death processes.

Experimental evidence of the predicted probability distribution was considered for a homogeneous population with a constant death rate. The results were inconclusive because the predicted variation was usually smaller than the expected variation due to errors of measurement and observation.

For a model with two viable states, statistical estimation of transition parameters was considered. The least squares estimators required the simultaneously solution of a complex system of non-linear equations. The methods of successive substitutions and the generalized Newton method were developed. Their application was successful for data simulated according to the derived probability distributions. But these techniques did not give convergence for data with deviations larger than predicted by the stochastic models.

Approved Carl W. Hall
Major Professor and
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SOME STOCHASTIC MODELS FOR MICROORGANISM DEATH KINETICS

By

Frederick Pierce Geyer

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PREFACE

The work reported is just a beginning. The topic has proven to be very interesting and it has provided a very rewarding experience in my graduate program. But many questions have arisen during the study that are not answered here.

The thesis topic and this dissertation are a direct result of the training received at the graduate level. I have tried to fully utilize this training in carrying out the dissertation research. To some, the approach may seem theoretical; but to others, the treatment will be at the applied level.

The first inspiration for a thesis on stochastic models came from Professor J. Gani (now at The University of Sheffield), whose classroom teaching ability initiated my interest in this topic. He was also instrumental in helping me formulate the two-stage death model (Chapter 4) and its solution.

The choice of consistent notation was difficult because of the extensive mathematical formulations. To allow for easy computer programming, alphanumeric characters were used for real and integer valued variables. In

so doing, the standard use of Greek symbols and English letters like i, j and k for transitions parameters was eliminated. In general, the letters i, j, k, l, m, and n were used to define only integer valued variables.

The Fortran listing of computer programs used is not included. Instead, the steps and procedures required in writing the programs are given. From my experience, the logic and notation used in Fortran programming is an individual matter and it takes about as much time to adopt someone else's program as to compose a new one. However, I shall be glad to supply a listing and/or Fortran source deck for the programs used in Chapter 6. These were especially difficult, and it took me several months to debug these programs.

I wish to express my appreciation to the many people in the department of Agricultural Engineering who have contributed to my graduate education. To Dr. D. R. Heldman, my research advisor, I am particularly indebted. His guidance and interest in the use of stochastic models were an abetment to my study. Also, Professors C. W. Hall and F. H. Buelow (now at The University of Wisconsin) were very helpful in guiding my graduate program.

Additional acknowledgment is given to Professor I. J. Pflug (Food Science) and Dr. D. Feldman (Statistics) for serving as guidance committee members. Finally, it seems

appropriate to acknowledge the support of my family who were a constant source of encouragement.

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NOMENCLATURE

c, c_1, etc.	arbitrary constants.
CV	coefficient of variation
E	event
exp(a)	e^a
g_1 and $g_1(t)$	transition rate, per unit time, from state 1 to state 3 (at time t)
h	death rate, per unit time
$\hat{h}$	death rate, per unit time
h_1 and $h_1(t)$	transition rate, per unit time, from state 1 to state 2 (at time t)
h_2 and $h_2(t)$	transition rate, per unit time, from state 2 to state 3 (at time t)
i	integer number
j	integer number
k	integer number representing total number in states 1 and 2
L_o	initial number in state 1
$\ell(t)$	number in state 1 at time t
M_o	initial number in state 2
m(t)	number in state 2 at time t
N_o	initial size of population
n(t)	size of population at time t
P	probability transition matrix
p	probability

$p_j(t)$	probability that there are j at time t
$p_{\ell,m}(t)$	probability that there are ℓ in state 1 and m in state 2 at time t
R and RN	a random number uniformly distributed on the interval 0,1
s(t)	survival probability for a organism at time t (2.25)
t	generally denotes time
u	generally denotes time
v	generally denotes time
X(t)	random variable with parameter t
Δ	small increment
$\psi(x,y,t)$	probability generating function with dummy variables x and y, and process variable t (4.18)
$\Omega(x,t)$	probability generating function with dummy variable x and process variable t (2.14)
σ^2	variance
σ	standard deviation
|	conditional probability
*	multiplication
$\binom{m}{n}$	$\frac{m!}{n!\,(m-n)!}$ if $m \geq n$, otherwise zero
	Bar above symbol indicates mean

CHAPTER 1

INTRODUCTION

Scope

The application of stochastic models to the death of populations of microorganisms is examined in this study. Several cases will be considered in detail. Emphasis will be given to mathematical derivations and statistical use of the models.

Mathematical models currently used for microbial death kinetics are deterministic; that is, for certain initial values and constant conditions, precise concentrations are predicted for subsequent times. Fluctuations about these precise values are assumed to be a result of extraneous experimental errors, and statistical methods are used as smoothing tools to achieve rate constants and population sizes.

Little attention has been given to the study of fluctuations arising inherently in the process itself. These fluctuations are caused not by errors, but by the laws of chance. If observations on a biological system are taken as elements in the set of all possible observations, these observations are usually elements of some specified subset of all possible observations. Stochastic

mathematical models create a subset that is more general and descriptive than that given by deterministic models. Allowance is made for random occurrence of individual events, and a probability is assigned to all elements within the created subset.

Development of Stochastic Processes

The mathematical understanding of physical and biological processes has continually broadened and deepened since the work of Newton in the 17th century. Models were sought that established correspondence between the main features of experimental results and abstract mathematical concepts. When randomness appeared in the phenomena, the classical theory of random variables was applied. Certain variables were treated as random variables in spaces of a finite number of dimensions.

In the 19th and 20th centuries, physical and biological problems arose involving the use of random variables in infinite-dimensional spaces. The mathematical framework developed for these problems became known as stochastic processes. A stochastic process has one or more varying parameters, of which time is the most common. The process may have a discrete or continuous varying parameter. For the discrete case, there may be a countable infinite number of possible values with a random variable for each value. A complete stochastic description will give a probability distribution of each

variable and all possible joint distributions. Likewise, for continuous variables, all variable distributions and joint distributions must be described.

Before the development of stochastic processes, physical and biological phenomena that developed with time were described with deterministic laws. Usually, chance was ignored and observations were considered predictable with a probability of one. However, in 1827, Robert Brown observed that particles in a liquid medium performed intense random movements. From these observations came studies leading to the Maxwell-Boltzman distribution for molecules. Soon probabilistic models were introduced into the kinetic theory of matter. These developments were utilized by Gibbs (1902) to lay the foundation of statistical mechanics.

Einstein (1905, 1906) and Smoluchowski (1906) showed that Brownian motion could be explained by assuming a tremendous number of irregular motions from molecules of the liquid. Later, Wiener (1923) gave a rigorous mathematical treatment of this model. In his version of Brownian motion, he showed that the displacement random variable is, with a probability equal to one, everywhere continuous as a function of t. This result is of major importance in the mathematical development of stochastic processes. With rather heuristic methods, Bachelier (1900) developed a probabilistic model for stock market operations. His

model was roughly equivalent to that of Einstein and Smoluchowski.

As early as 1908, in a different type of stochastic model, Erlang[1] carried out studies on telephone traffic problems. He derived the equilibrium form of the Kolmogorov equations for Markov processes with a countable number of states. If X(t) is a random variable denoting the number of events during time t, X(t) would be a discontinuous function of t, increasing by steps at times when events occur. When these events are the incoming calls at a telephone exchange, Erlang showed, under reasonable assumptions, that X(t) will have a Poisson distribution. His work became the foundation for probabilistic models of queueing problems.

Watson (1874) was the first to solve the problem of extinction of family surnames. This problem was a very early example of a stochastic process in discrete time. The name of Taylor (1920) could also be mentioned in this review of early work with stochastic models. He laid the foundation for the statistical theory of turbulence. In this problem, there is not only random variation with time, but also in space.

These are some of the prominent problems prior to 1925 involving random variation in time, space, etc.

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[1]See Brockmayer et al. (1948) for complete works of A. K. Erlang.

Unfortunately mathematical rigor was often lacking in these analyses. There was no general structure to cover all types of stochastic problems. With the exception of the work by Markov, the foundations for the mathematical theory of random processes were not laid till the late twenties and the thirties. Then Kolmogorov, Khintchine, Levy, Feller, and Doob contributed pioneering works. As interest has grown, many other authors have contributed to this field and applications have spread to nearly every branch of science.

Markov Process with Continuous Time Parameter

The most widely used type of stochastic process in physical and biological processes is known as Markov processes. Markov (1906) extended the range of probability theory from independent events to events that depend on the preceding trial. For a random variable X(t) with varying parameter t, a Markov process is defined by the conditional probability statement:

$$p\{X(t) = x | X(t_1) = x_1, X(t_2) = x_2, \ldots\ldots X(t_r) = x_r\}$$

$$= p\{X(t) = x | X(t_r) = x_r\} \qquad (1.1)$$

$$\text{for all } t \text{ and } t_1 < t_2 < \ldots\ldots < t_r < t \ .$$

Thus X(t) depends only on $X(t_r)$ and is independent of all previous values. Therefore, once the present state is

known, the future probabilistic behavior is uniquely determined. If the parameter intervals are discrete, the process is usually called a Markov chain.

For this study, stochastic processes in continuous time where the increments of X(t) correspond to non-overlapping time intervals are always mutually independent random variables. Such a process will satisfy the definition of a Markov process given above. In addition, if the probability distribution of the increment,

$$\Delta X(t) = X(t+\Delta t) - X(t), \tag{1.2}$$

depends only on the length t of the time interval, but is independent of the location of the interval or the time axis, the process is called stationary or homogeneous. Models for both homogeneous and non-homogeneous cases will be considered in this analysis.

A Markov process in continuous time with a finite number of states is of particular interest. For example, at any time a microorganism may be considered to be in one of two states: viable or non-viable.[2] For a Markov process in real time ($0 \leq t < \infty$) and a finite number of states labeled 1,2,...,N, the probability of being in state j at time t is labeled $p_j(t)$. From this definition, it follows that

$$\sum_{j=1}^{N} p_j(t) = 1 \tag{1.3}$$

for all t.

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[2] A viable organism is capable of reproduction when placed in a favorable environment.

The transition probabilities, $p_{ji}(t,s)$ can then be defined by

$$p_{ji}(t,s) = p\{X(t) = j \mid X(s) = i\}. \qquad (1.4)$$

$$s < t$$

X(t) or X(s) is the random variable representing the state of the system at time t or s whichever the case may be. If $p_i(s) > 0$, then the following two properties hold:

$$\text{Property 1} \qquad p_{ji}(t,s) \geq 0 \qquad (1.5)$$

$$\text{Property 2} \qquad \sum_{j=1}^{N} p_{ji}(t,s) = 1 \qquad (1.6)$$

The following special case of the Chapman-Kolmogorov equations can then be obtained:

$$p_{ki}(u,s) = \sum_{j=1}^{N} p_{kj}(u,t)\, p_{ji}(t,s) \qquad (1.7)$$

$$0 \leq s < t < u$$

Matrix notation may be used where P(t,s) is a N by N transition matrix. Equation 1.7 can be written

$$P(u,s) = P(u,t) * P(t,s) \qquad (1.8)$$

If the transition probabilities depend only on the difference t-s and not on the initial value, s, the

transition probabilities and matrix are stationary.

Equation 1.7 can be written

$$p_{ki}(s+t) = \sum_{j=1}^{N} p_{kj}(t)\, p_{ji}(s) \qquad (1.9)$$

$$s, t > 0$$

For matrix notation, this becomes

$$P(s+t) = P(t) * P(s) \qquad (1.10)$$

A detailed derivation is given in Appendix A.

The fundamental stochastic differential equations can then be derived from Equation 1.7. If u is replaced by $t + \Delta t$ and s by t in this equation, it can be differentiated (exact differential for a stationary model) with respect to t by taking the limit as $\Delta t \to 0$ after a few algebraic manipulations. This yields the Kolmogorov "forward system" of differential equations. On the other hand, if u is replaced by s and s by $s - \Delta s$, the differential may be obtained with respect to the variable s yielding the "backward system" of equations. Kolmogorov (1931) was the first to derive these two systems of equations. A more detailed derivation may be found in most stochastic texts such as Doob (1953, p. 235) and Feller (1957, p. 423).

Stochastic Population Models

The classical theories[3] of population growth treat the size of the population as a continuous variable that proceeds deterministically throughout the whole process. The fundamental assumption is that the future development of the population can be exactly predicted once the state at some initial point is completely specified.

Feller (1939) was the first to use the methods of Kolmogorov to treat population change as a Markov process in continuous time. His study led to a birth and death process with a discrete number of states. Much earlier, Yule (1924) had given a stochastic birth process in connection with the mathematical theory of evolution. He considered the creation of new species by mutation as a random event.

Feller's work was further developed by Arley (1943). He used a simple birth and death process in the stochastic theory of the "cascade showers" initiated by cosmic ray particles. In a series of papers, Kendall (1948a, 1948b, 1949) treated both homogeneous and non-homogeneous birth and death models. He also illustrated the use of generating functions for these processes. Following Kendall, many other authors have contributed to this field. Among the special cases considered is the inclusion of immigration into the population.

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[3]See Lotka, 1945, for a review of deterministic models of population size.

As a partial development of the birth and death models, the simple stochastic death model can be easily obtained. While early population studies were interested in processes that involved growth or mutations, death events were only included to make the model more realistic. Consequently, the study of population reduction with stochastic death models did not receive much consideration.

However, the concept that human death is a random phenomenon has a long history. Medieval artists often pictured death as something that "sooner or later" enslaved the individual. During the Great Plagues the mysterious ways in which death took the lives of many and left others untouched created a marked impression that death was an unpredictable event. Similarly, the concept of chance was identified with death as that which obeys no rule and defies all measure and prediction. But with the development of the scientific theory of probability, chance took on meaning as a measurable quantity. Pearson (1897) argued that human death statistics could be identified with probability distributions which could be defined in mathematical terms. From studies of human mortality statistics, he found five periods of human life that showed regular chance distribution of mortality.

In this section, individual deaths have been considered in a population where life was normal. That is, some individuals may die, but others live and perform the

usual functions of life. But in this study, the whole population is assumed under the stress of a lethal condition which inhibits the normal processes of growth and reproduction.

Ideas about the action of radiation on organisms have stimulated a large number of deterministic models[4] for the survival of organisms. These can usually be classed into two general types, "hit" and "target" models. "Hit" theory defines an event (death) as taking place when the organisms have received a determinable number of "hits" or quantities of radiation. "Target" theory extends this concept by theorizing that there are two or more targets, each of which must receive one or more hits for an event to occur.

Bharucha-Reid and Landan (1951) suggested a probability model for radiation damage. They theorized a chain of states with the ends being the absorbing states of death and complete recovery with immunity to further destruction. For hypothetical transition rates from one state to the next, the time dependent probabilities of reaching the two absorbing states are derived.

Hoffman (1957) postulated that death of individual cells was a random event, but he did not give any mathematical models. Recently, Fredrickson (1966a) has

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[4]See Zimmer (1960) for an extensive review with references for death models in radiation biology.

suggested the use of stochastic models to describe the killing of microorganisms. He gives a probability model of organism viability for three different cases. The first model is the simple death process for a homogeneous population. Geyer (1966) also illustrates this model for the death of microorganisms. The second model gives the time-dependent probability that a clump of organisms has one or more viable organisms remaining. In the third model, Fredrickson derives the stochastic equations from a model suggested by Johnson (1963). This model assumes that the spore contains at least one each of several different types of subcellular structures. The probability of a viable organism remaining is then obtained in terms of the destruction of all the different substructures.

The basic stochastic death model for microorganisms was also developed by Terui (1966). He used this model to predict the most probable time to kill a population of microorganisms. Some additional details of the work of Terui and Fredrickson will be given in Chapter 3 where the basic stochastic model is analyzed.

CHAPTER 2

ELEMENTARY STOCHASTIC MODEL

Fundamental Observations

Microorganism survival is currently considered to be reproducible according to deterministic laws. Fluctuation is ascribed, sometimes correctly and sometimes incorrectly, to experimental error. This study proposes that fluctuation in part is due to the random processes basic to the cause of death. And death kinetics for microorganisms are irreproducible processes.

The exact cause of microorganism death[1] is not known, but a number of rational explanations have been proposed for some lethal agents. If death is induced by moist heat, Rahn (1945, p. 39) has reasoned that death results from the denaturation of a single molecule. While other theories do not support this position, most agree that some unimolecular or complex molecular action occurs causing the loss of reproduction ability. If this is the case, the hypothesis of Bartholomay (1957, 1958) may be utilized. He argued that molecular processes are random processes.

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[1]Death is defined in the usual sense of nonviability when placed in a favorable environment.

To substantiate this proposition, Bartholomay considered the random implications of modern chemical reaction theory. From this viewpoint, randomness may be found in the Brownian-like motions of molecules, in the random intermolecular collisions, and in the accompanying intramolecular "random walks" from one discrete quantum energy level to another. Bartholomay (1962) has extended this line of reasoning to enzyme kinetics and concluded that stochastic models should be used for enzyme reactions. This conclusion can be relevant to the theory of Isaacs (1935) that explains cell death from disinfectants as a result of enzyme inactivation.

The action of chemical agents on microorganisms can also be considered as a molecular process. In some circumstances, the individual chemical particles execute Brownian motion with small, rapid steps in a random manner to penetrate and destroy the cell membrane.

In the case of irradiation, death may be caused by X-rays, gamma-rays, and alpha-rays from radioactive material, fast electrons (cathode rays and beta rays), and other fast charged particles produced by the use of accelerators. In all these, energy is transferred in discrete quantities, and the time between emissions is a random variable. Rutherford et al. (1931) observed this randomness early in this century with radioactive material. The waiting times between decompositions are often described with a negative exponential distribution and the number of emissions by a Poisson distribution. In addition, the spatial distribution

of the radiation in the medium containing the cells can also be considered a random variable. The absorption of radiation by organisms has been theorized to have effects such as local or point energy release, molecular transformations following quantum jumps, polarization, separation of charge and production of free organic radicals (Zimmer, 1960, p. 15). These actions collectively or singly are a consequence of statistical properties of the organisms and of the basic constitution of matter.

Without further consideration of the causes of microorganism death, the evidence from the several cases considered indicate that one or more random factors contribute to all death processes. The modern theory of quantum mechanics establishes a comprehensive foundation that there is a basic physical randomness of molecular motion within all organized protoplasm. But an exact or deterministic relationship may appear on the macroscopic scale. This gives an illusion that the process is reproducible. Usually, the instrumentation lacks the sensitivity to measure fluctuation on the microscopic scale. On the other hand, the growth of microorganism populations can be observed accurately. This is possible by measuring the time intervals between cell divisions with a microscope (Kelly, 1932). But since death is not an observable event such as cell division, better instrumentation is required to appraise the occurrence of individual deaths.

Derivation of Elementary Model

This section describes the complete derivation of a non-homogeneous death process. It illustrates the mathematical techniques used in this study and presents the derivation for the time dependent case. Derivations for the homogeneous process may be found elsewhere. For example, Bailey (1964, p. 90) considers the homogeneous death process.

A lethal environment will be assumed and not specified as to whether it results from heat, chemical poisons, irradiation, etc. The lethal condition is applied at time zero with uniform intensity throughout the initial population of organisms. In order to derive the mathematical model for the population during the process, the following axioms are accepted.

1. A probability parameter h(t) is defined so that the probability of death for any organism during a short interval t, t+Δt is h(t+$\phi\Delta$t). The function h(t) represents the death rate of the organism at time t. And ϕ is chosen so that:

$$\frac{1}{\Delta t}\int_{t}^{t+\Delta t} h(\tau)d\tau = h(t+\phi\Delta t) \qquad (2.1)$$

The death rate may be defined in terms of physical and chemical properties of the organism and the environment, but this relationship need not be established to derive the general stochastic model.

2. The probability of more than one death during the interval t, t+Δt is $o(\Delta t)$ where $o(\Delta t)$ is the zero order of Δt. That is, $o(\Delta t)$ is some function of Δt such that the limit of $\frac{o(\Delta t)}{\Delta t}$ as Δt approaches zero is zero.
3. The joint occurrence of events occurring in non-overlapping time intervals is statistically independent. Thus, the probability of two or more of these events is calculated by multiplying together the probabilities for each.

Axiom 1 implies that the deaths of cells are independent events. Starting with an initial population N_o, let n(t) be the random variable representing the number of viable cells at time t. Note that n(t) is a time dependent discrete random variable with a finite number of states. The probability of having n living organsism at time t is designated $p_n(t)$. This probability is not directly obtainable, but it can be derived from the stochastic differential-difference equations (the Kolmogorov equations). The "forward system" of these equations may be obtained from consideration of $p_n(t+\Delta t)$. This probability can be obtained by applying Equation 1.7 of Chapter 1.

First, consider all events leading to n organisms at time $t+\Delta t$. Three mutually exclusive events (E_1, E_2, E_3) may produce this condition. They are described as follows:

E_1: The joint occurrence of events E_{11} and E_{12}.

E_{11}: $N_o - n + 1$ deaths occur in time $t(o,t)$. By definition this probability is $p_{n+1}(t)$.

E_{12}: One death occurs among the $n + 1$ organisms in time Δt. According to axiom 1, this probability is

$$(n+1)h(t+\phi\Delta t)\Delta t$$

Therefore, according to axiom 3 the probability of E_1 is given by the equation

$$p\{E_1\} = p\{E_{11}\}p\{E_{12}\} = p_{n+1}(t)\ (n+1)h(t+\phi\Delta t)\Delta t \qquad (2.2)$$

E_2: The joint occurrence of events E_{21} and E_{22}.

E_{21}: $N_o - n + i$ $(i \geq 2)$ deaths occur during time t. This probability is $p_{n+i}(t)$.

E_{22}: During t, i $(i \geq 2)$ deaths occur among the $n + i$ organisms. According to axiom 2, this probability is $o(\Delta t)$. Thus,

$$p\{E_2\} = p\{E_{21}\}p\{E_{22}\} = \sum_{i=2} p_{n+i}(t)\ o(\Delta t) \qquad (2.3)$$

E_3: The joint occurrence of events E_{31} and E_{32}

E_{31}: N_o - n deaths take place during time t. This probability is $p_n(t)$.

E_{32}: No deaths occur for the n remaining organisms during Δt. Since the probability of one or more deaths is $nh(t+\phi\Delta t)\Delta t + o(\Delta t)$, the probability of no deaths is $1 - nh(t+\Delta t)\Delta t - o(\Delta t)$. According to axiom 3,

$$p\{E_3\} = p\{E_{31}\}p\{E_{32}\} = p_n(t)\left[1 - nh(t+\phi\Delta t)\Delta t - o(\Delta t)\right] \quad (2.4)$$

Since events E_1, E_2 and E_3 are mutually exclusive ways in which $p_n(t+\Delta t)$ may occur, the probability for each of the three events is summed,

$$p_n(t+\Delta t) = p\{E_1\} + p\{E_2\} + p\{E_3\} \quad (2.5)$$

Substituting the expressions obtained for the terms on the right side of this equation,

$$p_n(t+\Delta t) = p_{n+1}(t)(n+1)h(t+\phi\Delta t) + \sum_{i=2} p_{n+i}(t)o(\Delta t)$$

$$+ p_n(t)\left[1 - nh(t+\phi\Delta t)\Delta t - o(\Delta t)\right] \quad (2.6)$$

By subtracting $p_n(t)$ from both sides of this equation and dividing by Δt, it becomes:

$$\frac{p_n(t+\Delta t) - p_n(t)}{\Delta t} = p_{n+1}(t)(n+1)h(t+\phi\Delta t) + \sum_{i=2} p_{n+i}(t) \frac{o(\Delta t)}{\Delta t} - p_n(t)\, n\, h(t+\phi\Delta t) \qquad (2.7)$$

Now consider the limit as Δt tends to zero, the left side of 2.7 becomes the derivative $\frac{d\, p_n(t)}{dt}$. By axiom 2, the value of $\frac{o(\Delta t)}{\Delta t}$ goes to zero, and $h(t+\phi\Delta t)$ becomes $h(t)$. Consequently, Equation 2.7 can be written

$$\frac{d\, p_n(t)}{dt} = p_{n+1}(t)(n+1)h(t) - p_n(t)\, n\, h(t) \qquad (2.8)$$

where $n = 0,1,\ldots,N_o$

Equation 2.8 represents a system of $N_o + 1$ equations. Each will have two terms on the right side of 2.8 except for the case where n is 0 or N_o. Since the state $N_o + 1$ can not exist, $p_{N_o+1}(t)$ has value zero. Therefore Equation 2.8 is reduced to the following equation for $n = N_o$.

$$\frac{d\, p_{N_o}(t)}{dt} = p_{N_o}(t)\, N_o h(t) \qquad (2.9)$$

And if $n = 0$, Equation 2.8 yields

$$\frac{d\, p_o(t)}{dt} = p_1(t)\, h(t) \qquad (2.10)$$

The whole system of equations represented by 2.8 can be efficiently represented by the matrix notation:

$$\frac{dP(t)}{dt} = h(t)\ A\ P(t) \qquad (2.11)$$

where P(t) is the vector $\left[p_{N_o}(t),\ p_{N_o-1}(t),\ \quad p_1(t),\ p_o(t)\right]$ and A is the N_o+1 by N_o+1 matrix

$$\begin{bmatrix} -N_o & & & & & & \\ N_o & -(N_o-1) & & & & & \\ & (N_o-1) & -(N_o-2) & & & & \\ & & & & & & \\ & & & & 3 & -2 & \\ & & & & & 2 & -1 \\ & & & & & & 1 \quad 0 \end{bmatrix}$$

Solution of the System of Differential Equations

The system of differential equations derived in the previous section may be solved in several ways. Provided h(t) is defined, these equations can be integrated successively starting with $n = N_o$. This process will yield the general solution:

$$p_n(t) = \exp(-n \int_o^t h(\tau)d\tau)\Big[(n+1) \int_o^t h(\tau)p_{n+1}(\tau)$$

$$\exp(n \int_o^t h(\tau)d\tau)d\tau + c_n\Big] \qquad (2.12)$$

c_n is the constant of integration defined by initial conditions ($p_{N_o}(o) = 1$; $p_n(o) = o$, $n < N_o$). But this method is very laborious, especially since N_o is usually very large. Also, the integration required in 2.12 could not be carried out without defining h(t). The latter difficulty is avoided when the matrix form is considered. For this approach, the solution of Equation 2.11 is easily obtained as

$$P(t) = \exp(A \int_o^t h(\tau)d\tau) \qquad (2.13)$$

The form of this solution is simple, but the evaluation of $\exp(A \int_o^t h(\tau)d\tau)$ is a long and difficult process unless the eigenvalues and eigenvectors of A can be determined easily.

The most appropriate method for solving a system of Kolmogorov difference equations such as 2.8 is to use a generating function. With generating functions, a system of differential equations can usually be reduced to a partial differential equation. In addition, the moments of the probability distribution are easy to obtain when the generating function is known. To solve the system of equations represented in 2.8, the following generating function $\Omega(x,t)$ is defined.

$$\Omega(x,t) = \sum_{n=0}^{N_o} x^n p_n(t) \qquad (2.14)$$

From this definition, the following two partial differential equations are derived.

$$\frac{\partial \Omega(x,t)}{\partial t} = \sum_{n=0}^{N_o} x^n \frac{dP_n(t)}{dt} \tag{2.15}$$

$$\frac{\partial \Omega(x,t)}{\partial x} = \sum_{n=0}^{N_o} nx^{n-1} p_n(t) \tag{2.16}$$

If Equation 2.8 is multiplied by x^n and summed over all values of n, it becomes

$$\sum_{n=0}^{N_o} x^n \frac{dp_n(t)}{dt} = \sum_{n=0}^{N_o} (n+1)\, x^n h(t) p_{n+1}(t)$$

$$- \sum_{n=0}^{N_o} n\, x^n\, h(t) p_n(t) \tag{2.17}$$

This equation can be rewritten in a more suitable form by shift of axis to discard meaningless terms.

$$\sum_{n=0}^{N_o} x^n \frac{dp_n(t)}{dt} = \sum_{n=0}^{N_o} n\, x^{n-1}\, h(t) p_n(t)$$

$$- x \sum_{n=0}^{N_o} nx^{n-1} h(t) p_n(t) \tag{2.18}$$

Equations 2.15 and 2.16 can then be substituted into 2.18 and a partial differential equation involving the generating function is obtained.

$$\frac{\partial \Omega(x,t)}{\partial t} = h(t)\ (1 - x)\ \frac{\partial \Omega(x,t)}{\partial x} \tag{2.19}$$

Using Lagrange's method of auxiliary equations as described in Appendix B, the following ordinary differentials have the same solution as 2.19.

$$\frac{d\Omega(x,t)}{0} = \frac{dx}{h(t)(1 - x)} = \frac{dt}{-1} \tag{2.20}$$

Two independent solutions of 2.20 are

$$\Omega(x,t) = c_1, \tag{2.21}$$

and

$$(x - 1)\ \exp\left(-\int_0^t h(\tau)d\tau\right) = c_2 \tag{2.22}$$

with c_1 and c_2 arbitrary constants. Therefore, $\Omega(x,t)$ is some function of $(x - 1)\exp\left(-\int_0^t h(\tau)d\tau\right)$. For the initial conditions, $\psi(x,o) = x^{N_o}$, the general solution is

$$\Omega(x,t) = \left[x\ \exp\left(-\int_0^t h(\tau)d\tau\right) + 1 - \exp\left(-\int_0^t h(\tau)d\tau\right)\right]^{N_o} \tag{2.23}$$

By a series expansion of 2.23, the coefficients of x^n are obtained. From the definition of the probability generating function, these coefficients are the values of $p_n(t)$. Thus,

$$p_n(t) = \binom{N_o}{n} s(t)^n [1 - s(t)]^{N-n} \tag{2.24}$$

where $$s(t) = \exp(- \int_o^t h(\tau)d\tau) \tag{2.25}$$

and $$\binom{N_o}{n} = \frac{N_o!}{(N_o-n)!\ n!} \quad .$$

This result could be obtained directly from 2.23 by observing that it is a generating function for a binomial distribution with parameter s(t). Since s(t) is the survival probability for any organism in the population and each organism was assumed independent, the binomial distribution of Equation 2.24 can be obtained from Equation 2.9 for a N_o of one. However, the method of solution given illustrates the techniques used for more complex models (Chapter 4) that can not be solved by simple methods.

The distribution of the random variable n(t) can be used to determine common statistics such as the mean $\bar{n}$ and variance σ^2. These two statistics can be obtained directly from the probability generating function by applying the following formulas.

$$\bar{n}(t) = \psi'(1,t) = N_o \exp(- \int_o^t h(\tau)d\tau) \qquad (2.26)$$

$$\sigma^2(t) = \psi''(1,t) + \psi'(1,t) - [\psi'(1,t)]^2$$

$$= N_o \exp(- \int_o^t h(\tau)d\tau)[1 - \exp(- \int_o^t h(\tau)d\tau)] \qquad (2.27)$$

Next, the distribution function of the arrival time of an event may be obtained. Starting with N_o organisms, the probability that at time t no event has occurred is given by 2.24 for $n = N_o$. Accordingly,

$$p_{N_o}(t) = \exp(- N_o \int_o^t h(\tau)d\tau) \qquad (2.28)$$

This is also the probability that the first event happens at some instant greater than t. Therefore, the distribution function of the arrival time u of the first event is given by

$$F(u) = 1 - \exp(- N_o \int_o^u h(\tau)d\tau) \qquad (2.29)$$

and the corresponding density function is

$$f(u) = F'(u) = N_o h(u) \exp(- N_o \int_o^u h(\tau)d\tau) \qquad (2.30)$$

where $h(\tau) \geq 0$ for $\tau(0,\infty)$ and $h(\tau) = 0$ for only a finite interval. This equation can be used to give the distribution of the time interval between any two successive events if the value of N_o is adjusted to the level of the

population and the time scale and $h(\tau)$ are shifted to the point the last event occurred.

The deterministic model of this process may be compared to the stochastic in several ways. Deterministic kinetics are based on the Law of Mass Action. Accordingly, any change in population is proportional to the size of the population. The ordinary differential equation

$$\frac{dn}{dt} = -n\,\hat{h}(t) \tag{2.31}$$

describes the model. In this case, the population is treated as a continuous function of time, although n is discontinuous for a real death process. Equation 2.31 may be integrated to give

$$n(t) = N_o \exp\left(-\int_o^t \hat{h}(\tau)d\tau\right) \tag{2.32}$$

This equation has the same form as the equation for the mean of the stochastic model (2.26), therefore $n(t)$ and $\hat{h}(t)$ are the deterministic equivalent of $\bar{n}(t)$ and $h(t)$ in the stochastic model. Thus, the stochastic model is "consistent in the mean" with the deterministic model. Consequently, the deterministic model may be considered a special case of the stochastic model. The stochastic model would yield the same result as the deterministic only if a large number of cases were averaged.

The stochastic model not only predicts a fluctuation from the mean, but it specifies the expected size of these deviations. With this model, the reproducibility of the process can only be considered as a joint probability distribution of two or more independent events whose probabilities are specified by Equation 2.24. However, it is possible to specify a range of values within which the process would be expected to lie for any level of significance desired.

Because the probability distribution derived in Equation 2.24 is binomial, it may be approximated with a normal distribution according to the de Moivre-Laplace Limit theorem (Feller, 1957, Chapter 7). The error of this approximation will be small if the variance is large. If the variance is small, a Poisson distribution could be used as an approximation of the distribution because the variance will be small in the same intervals where the probability is very small or close to one. For a normal approximation, about 68 percent, 95.5 percent and 99.7 percent of the distribution would be expected to fall within one, two, and three standard deviations, respectively of the mean.

CHAPTER 3

HOMOGENEOUS CASE OF ELEMENTARY MODEL

Description

If the organism death rate h(t) is independent of time t, the process is considered homogeneous and h(t) = h. Assuming a constant lethal environment, several microorganism death processes exhibit homogeneous characteristics. Rahn (1945) and Stumbo (1965) both concluded that the death rate of spores by constant temperature heat inactivation is independent of time. Their conclusion was based on their own laboratory studies as well as those by other researchers.

On the contrary, considerable evidence has been obtained that heat inactivation of spores is time dependent for some conditions. For example, Frank (1957) and Humphrey (1961) have documented time dependent cases. For spore irradiation death processes, neither the homogeneous nor the non-homogeneous elementary model seems appropriate. Instead the process is considered more complex, and a "target" or "hit" model (see p. 11, Chapter 1) is usually given.

The homogeneous case of heat inactivation will be non-homogeneous if the temperature is not held constant. Under these conditions, the death rate is a function of temperature.

Assuming that a death process is homogeneous, its probability distribution may be easily obtained from the non-homogeneous stochastic model in Chapter 2 (Equation 2.22). Consequently,

$$p_n(t) = \binom{N_o}{n} \exp(-nht)\left[1 - \exp(-ht)\right]^{N_o-n} \qquad (3.1)$$

$$\bar{n}(t) = N_o \exp(-ht) \qquad (3.2)$$

$$\sigma^2(t) = N_o \exp(-ht)\left[1 - \exp(-ht)\right] \qquad (3.3)$$

Bailey (1964, p. 91), and Frederickson (1966a) gave this distribution for a stochastic model of microorganism death kinetics. As an example of this type of process, Figure 3.1 shows a hypothetical death process for the distribution of Equation 3.1. As is commonly done, the log of the number of survivors is plotted with a linear time scale. Thus, the mean is a straight line. To extend this consideration to some of the unique features of the homogeneous model and its application, the following was developed.

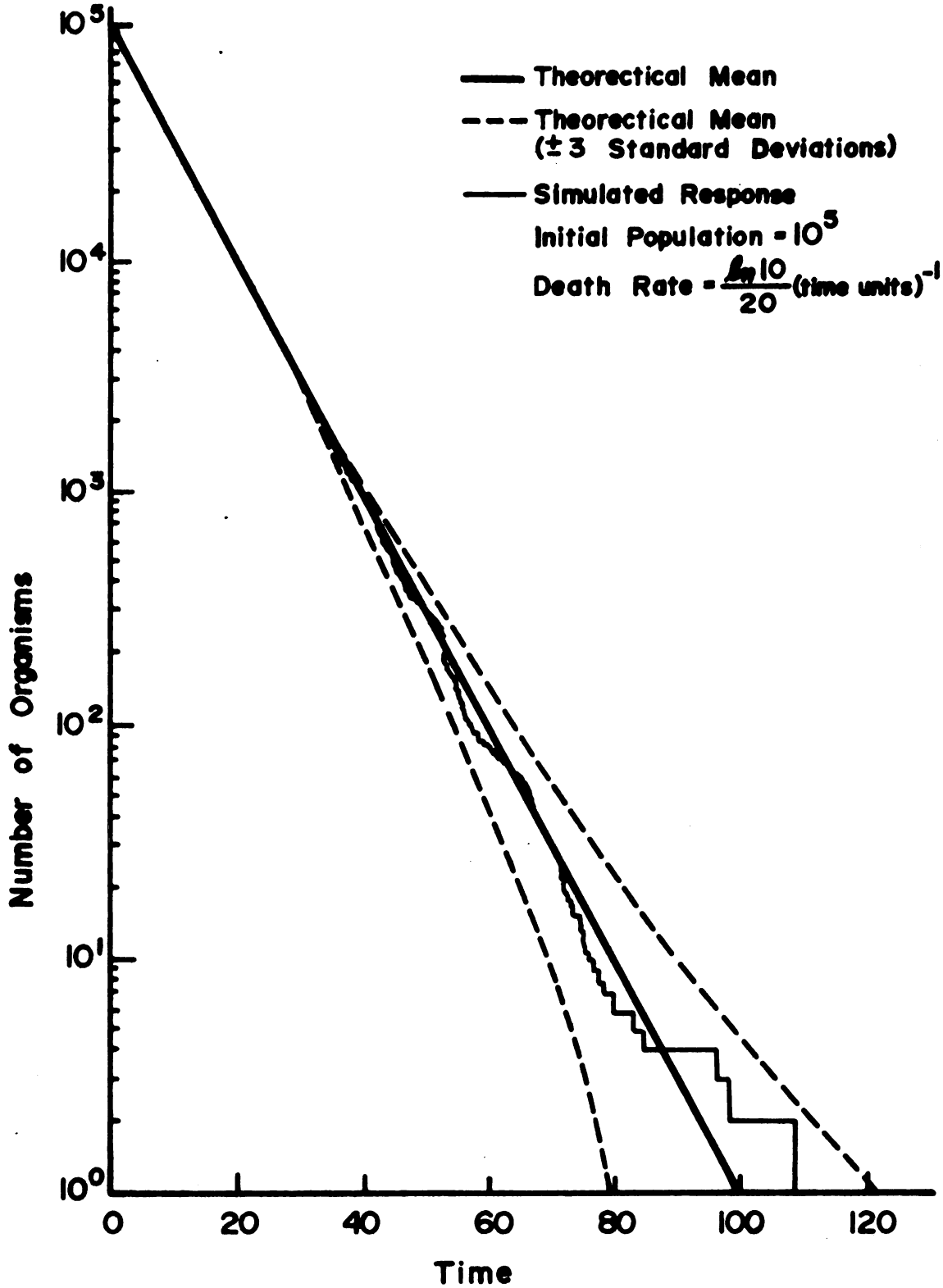

Figure 3.1.--Survival Curve for An Elementary Death Process.

First, consider the variance of the process. The lines in Figure 3.1 showing the mean plus and minus three standard deviations may be deceptive. The time of maximum variance or standard deviation is not evident from this figure. Since the variance (defined in 3.3) is a continuous function with value zero at the ends of the time scale $(0,\infty)$, a time t_m of maximum variance may be found for $0 < t_m < \infty$.

The derivative of 3.3 with respect to time and solved for t in the usual manner to find a maximum yields:

$$t_m = \frac{\ell n\ 2}{h} \tag{3.4}$$

The size of the maximum variance σ_m^2 is then:

$$\sigma_m^2 = \frac{N_o}{4} \tag{3.5}$$

At time t_m, the mean is $\frac{N_o}{2}$ or the expected population size is half that of the original population. Also, the time of maximum variance is solely a function of the death rate constant and the size of the maximum variance is only a function of initial population size.

Considering another statistic of the process, the coefficient of variation, CV, is a continuously increasing function of time since

$$CV(t) = \frac{\sigma(t)}{\bar{n}(t)} = \sqrt{\frac{\exp(ht) - 1}{N_o}} \quad . \tag{3.6}$$

This statistic may be thought of as a measure of dispersion relative to the mean while the variance is an absolute measure of the irreproducibility of the process.

On the other hand, the ratio of the variance to the mean approaches unity as t becomes large, as shown by

$$\frac{\sigma^2(t)}{\bar{n}(t)} = 1 - \exp(-ht) \tag{3.7}$$

Hence, the variance and the mean are approximately equal for large values of t. Since the Poisson distribution will approximate the binomial distribution for large t, the above condition is obvious.

The distribution function F(u) of time u between death events is easy to obtain for the homogeneous case. From Equation 2.29

$$F(u) = 1 - \exp(-N'hu) \tag{3.8}$$

where N' is the size of the population before a death occurs. Hence, the density function is the negative exponential and given by

$$f(u) = N'h \exp(-N'hu) \tag{3.9}$$

If N' is one, Equation 3.9 gives the density function of an individual lifetime. Since all individual organisms are considered independent, the individual lifetimes v

for the whole population has this distribution. This can be written

$$f(v) = h \exp(-hv) \tag{3.10}$$

These distributions may be used to derive the expected or mean time to reduce the population to some specific level. Using the density given in Equation 3.9, the expected time interval to reduce an initial population, N_o, by one is given by

$$\int_o^\infty uf(u) = \frac{1}{h\ N_o} \tag{3.11}$$

By the same method the expected time period to reduce the population by one more is $\frac{1}{h(N_o - 1)}$. Continuing this procedure, the expected time, $\bar{t}_o$, to reduce an initial population, N_o, to the zero level is the sum of all mean times for each individual reduction. By making this addition,

$$\bar{t}_o = \frac{1}{h} \sum_{i=1}^{N_o} \frac{1}{i} \tag{3.12}$$

A computer calculation could be used to determine $\bar{t}_o$ for large N_o. Terui (1966) showed an easy method to approximate this value. Using Euler's constant,[1] he found

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[1]Euler's constant is the limit as $m \to \infty$ of $\left(\sum_{i=1}^{m} \frac{1}{i} - \log_e m \right)$. See Abramowitz and Stegun (1964, p. 255).

$$\bar{t}_o \simeq \frac{1}{h}(\log_e N_o + 0.577). \qquad (3.13)$$

Simulation of Process

Using the probability distribution for the homogeneous process, data may be generated by simulation techniques. Two methods of simulation were considered. First, a population destruction as a step-by-step process was obtained. Second, a data value was generated given any point in time of the process. This latter method is used to simulate laboratory procedures. For experimental work, the only way to count the number present is to disrupt the death process and determine the number of organisms using standard microbiological techniques. Thus, one population can only give one data point. Consequently, a large number of homogeneous samples are required to obtain a number of data points for the process.

To simulate the first type of process, assume a computer is available with a library function to generate random numbers with a uniform distribution over the interval (0,1). According to the axioms of the general stochastic model in Chapter 2 (p. 16), the probability of a death during a short interval (t, t+Δt) is

$$p_D = n\ h\ \Delta t \qquad (3.14)$$

where n is the number of organisms at time t. The error

of this equation is proportional to $o(\Delta t)$. If Δt is sufficiently small, $o(\Delta t)$ will be insignificant.

To simulate a process starting with an initial number of organisms at time zero, a computer program was written to do the following:

1. Calculate the probability of a death p_D according to Equation 3.14.
2. Generate a random number RN with uniform distribution (0,1).
3. Increase time by Δt.
4. Check if RN is equal to or less than p_D. If this condition is not met, repeat the above procedure starting at step 2. If RN is equal to or less than p_o, perform step 5.
5. Decrease n by one, note time and size of population. Then repeat the above steps starting at step 1, and continue until the population reaches zero.

An example of this simulation is given in Figure 3.2.

Another method to make a step-by-step simulation of the process may be derived without any approximation as in the preceding method. Using the distribution function for the time interval between events given in Equation 3.8, simulated time intervals v may be generated. Assuming a random number, R, with a uniform distribution (0,1) can be obtained, then the desired simulated time interval, v, is determined from Equation 3.8 where F(u)

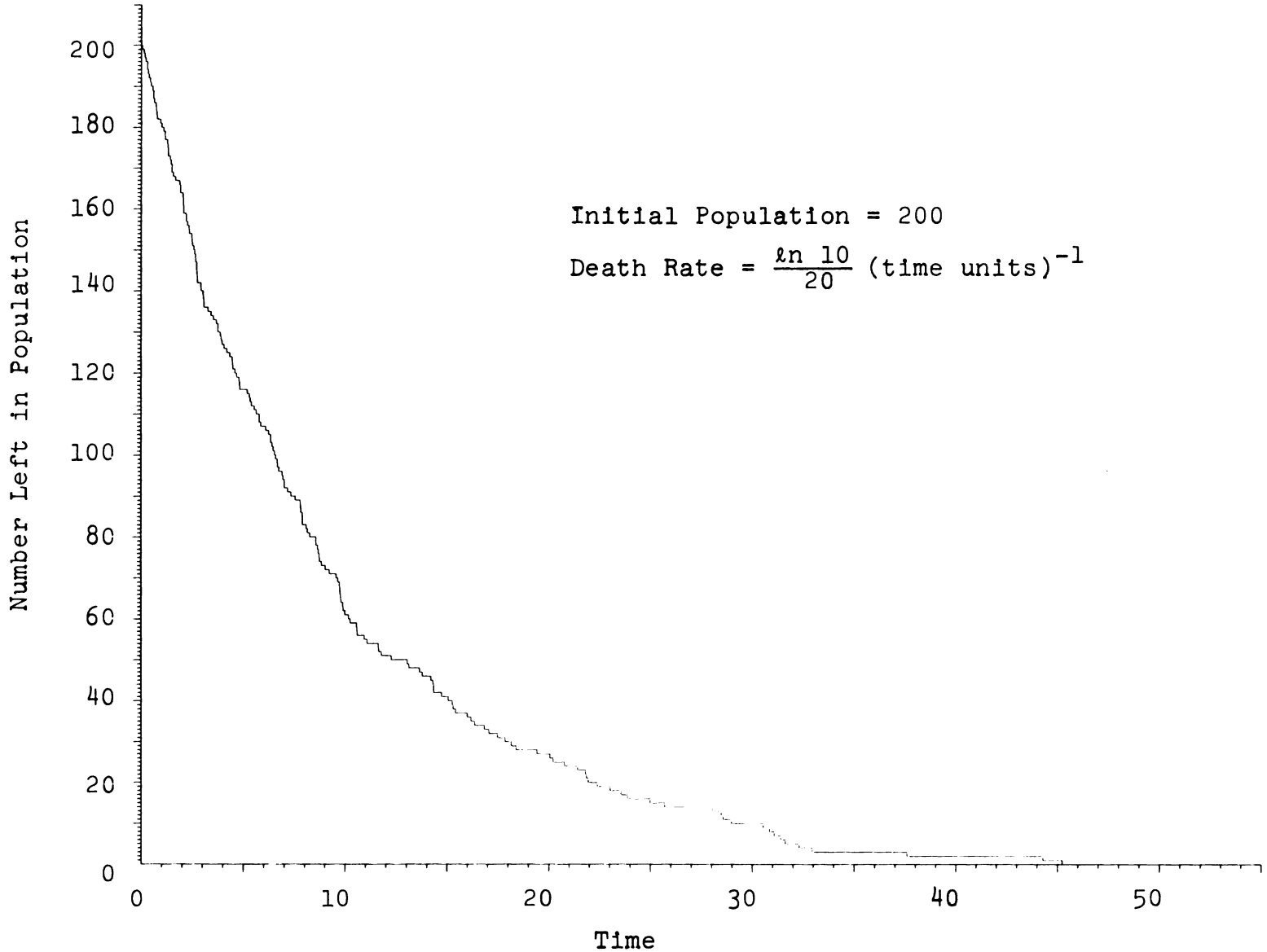

Figure 3.2.--Simulated Death Process Using Equation 3.14.

is replaced by R. Shreider (1964, p. 252) outlines this method and its proof. Thus,

$$v = -\frac{1}{n} \log_e(1-R). \tag{3.15}$$

Since 1-R is also uniform (0,1), the above equation can be reduced to

$$v = -\frac{1}{n} \log_e(R). \tag{3.16}$$

Simulation of the process was then achieved with a computer program designed to perform the following:

1. Initialize the program with N_o organisms and time equal to zero.
2. Calculate v according to Equation 3.16.
3. Increase time by v and decrease n by one.
4. Record time and population size. Then repeat the above steps starting at step 2 until the population reaches zero.

An example of this type of simulation is given in Figure 3.3. The difference between the curve of Figure 3.2 and that of Figure 3.3 is caused by the random variables generated by the computer programs, and not by difference in the methods used to obtain these two curves.

To simulate laboratory data for a process starting with a number of samples, a different technique can be

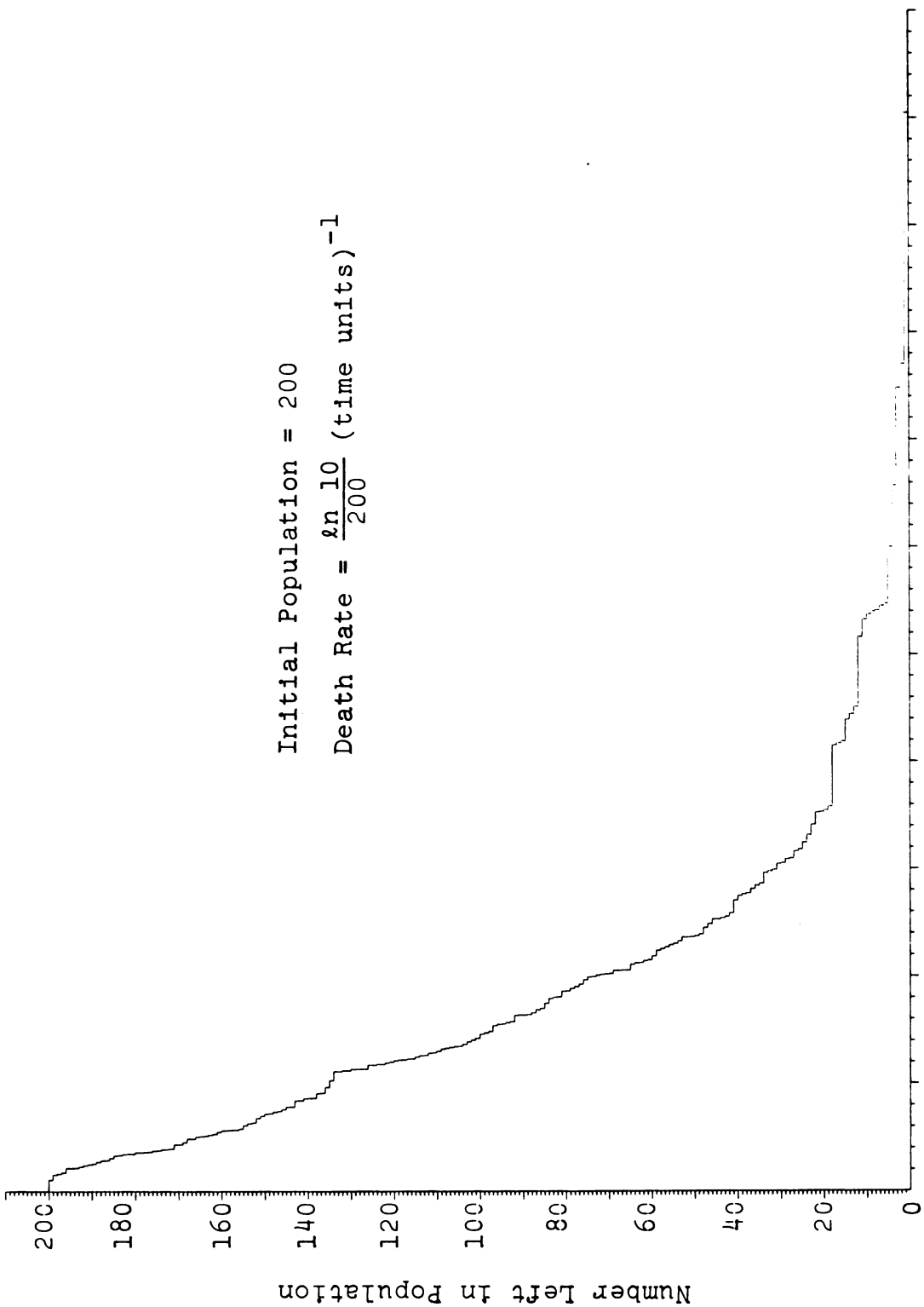

Figure 3.3.--Simulated Death Process by Generation of Time Intervals Between Each Death (Equation 3.16).

used. Since each experimental sample is independent of the others and provides one data point, each simulated data point may be simulated independently. The problem is then reduced to simulating only one point for any time given the initial concentration and death rate. This point will have the binomial distribution given by Equation 3.1.

The generation of random numbers with a known distribution is not difficult. Shreider (1964, p. 252) gives the procedure for continuous density functions. But this technique can be extended to the discrete case in the following manner. In order to obtain a number belonging to a set of random numbers, n_i, having the probability mass function p_n, generate a random number R with uniform distribution (0,1). Then choose the smallest n_i such that

$$\sum_{j=0}^{n_i} p_j \geq R \tag{3.17}$$

This method may be simplified. Since p_j is binomial, it may be approximated by a normal distribution. To generate a normal distribution, again assume that random numbers with uniform distribution (0,1) may be acquired without difficulty. Then a random variable, V, with a normal distribution (0,1) may be easily generated by the following equation derived by Box and Muller (1958).

$$V = (-2 \log_e R_1)^{1/2} \cos(2\pi R_2) \qquad (3.18)$$

where R_1 and R_2 are random numbers with uniform distribution (0,1). The cosine function in the above equation may be interchanged with the sine function without changing the distribution of V. Since V is normal (0,1), the required random number, n_r, may be determined using the values of the mean and variance given in Equations 3.2 and 3.3. Thus,

$$n_r(t) = \bar{n}(t) + V^*\sigma(t) \qquad (3.19)$$

An example of data generated using this procedure is given in Figure 3.4.

A Comparison of Deterministic and Stochastic Models

The deterministic model has at least two important faults. First, it assumes the population size is a real-valued continuous function of time rather than an integer-valued function of time. Second, it assumes that the population size at any given time will always be the same if the initial conditions are not changed. The second fault is the more objectionable of the two because it ignores intrinsic random factors that may influence the destruction of microorganisms.

The stochastic approach questions the assumptions of the deterministic model and thus its validity. By

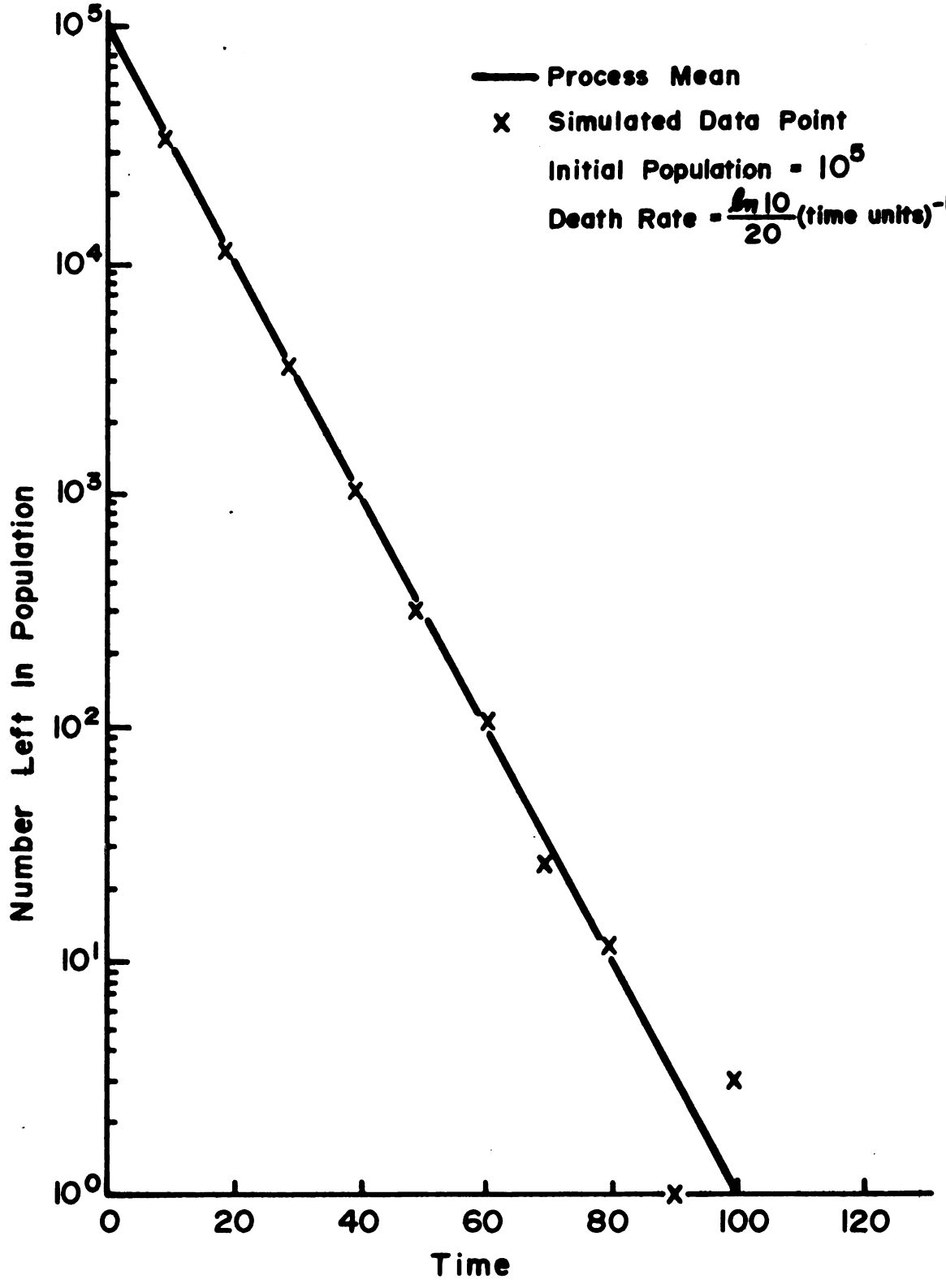

Figure 3.4.--Simulated Experimental Data For Elementary Death Process.

substituting probability relationships for deterministic ones, random fluctuation is expected even in the total absence of experimental irregularities. However, the amount of inherent variability may be small and unmeasurable by experimental procedures. As shown in Figure 3.1 the expected range of most of the predicted variability is too small to be detected for the reduction of the first half of the population. On the other hand, the predicted fluctuations become fairly large relative to the mean as the population grows small.

The difference between the stochastic and deterministic equations may be compared for prediction of process times required for sterility of all organisms in a population. To obtain complete sterility, the viable population must be reduced to zero. For the deterministic model, the population only reaches zero as time approaches infinity. However, this model may be used to predict practical process times if the value of the mean is taken to represent the probability of viability for the whole population. To use this approximation, the mean must have a value less than one. Then the probability of any viable organisms remaining at time, t, is designated q in the following equation.

$$q = N_o \exp(-ht) \qquad (3.20)$$

where $q < 1$

Solving this equation for t, the process time to obtain a probability of viable population, q, is

$$\text{Process Time (Deterministic Model)} = -\frac{1}{h}\,\ell n\left(\frac{q}{N_o}\right) \qquad (3.21)$$

$$q < 1$$

For the stochastic model, q is $1 - p_o(t)$ by definition. Using the value of $p_o(t)$ given in Equation 3.1, the process time required may be specified as follows.

$$\text{Process Time (Stochastic Model)} = -\frac{1}{h}\,\ell n\left(1 - (1-q)^{N_o^{-1}}\right) \qquad (3.22)$$

To compare these two predictions, Figure 3.5 shows a plot of both for an initial population of 10^5. By using semi-logarithmic scales, the deterministic prediction is a straight line. This line does not take on any values for the initial time interval because the mean of the process must be reduced to one before the process time can be determined (Equation 3.21). This line can be considered as a continuation of the mean line in Figure 3.1.[1] On the other hand, the stochastic model gives a probability of viability for all points in time. It yields a line that asymptotically approaches the deterministic curve as the process transpires. By the time the probability of any

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[1]The time scale in Figure 3.5 has been changed from that of Figure 3.1, but the initial population is the same.

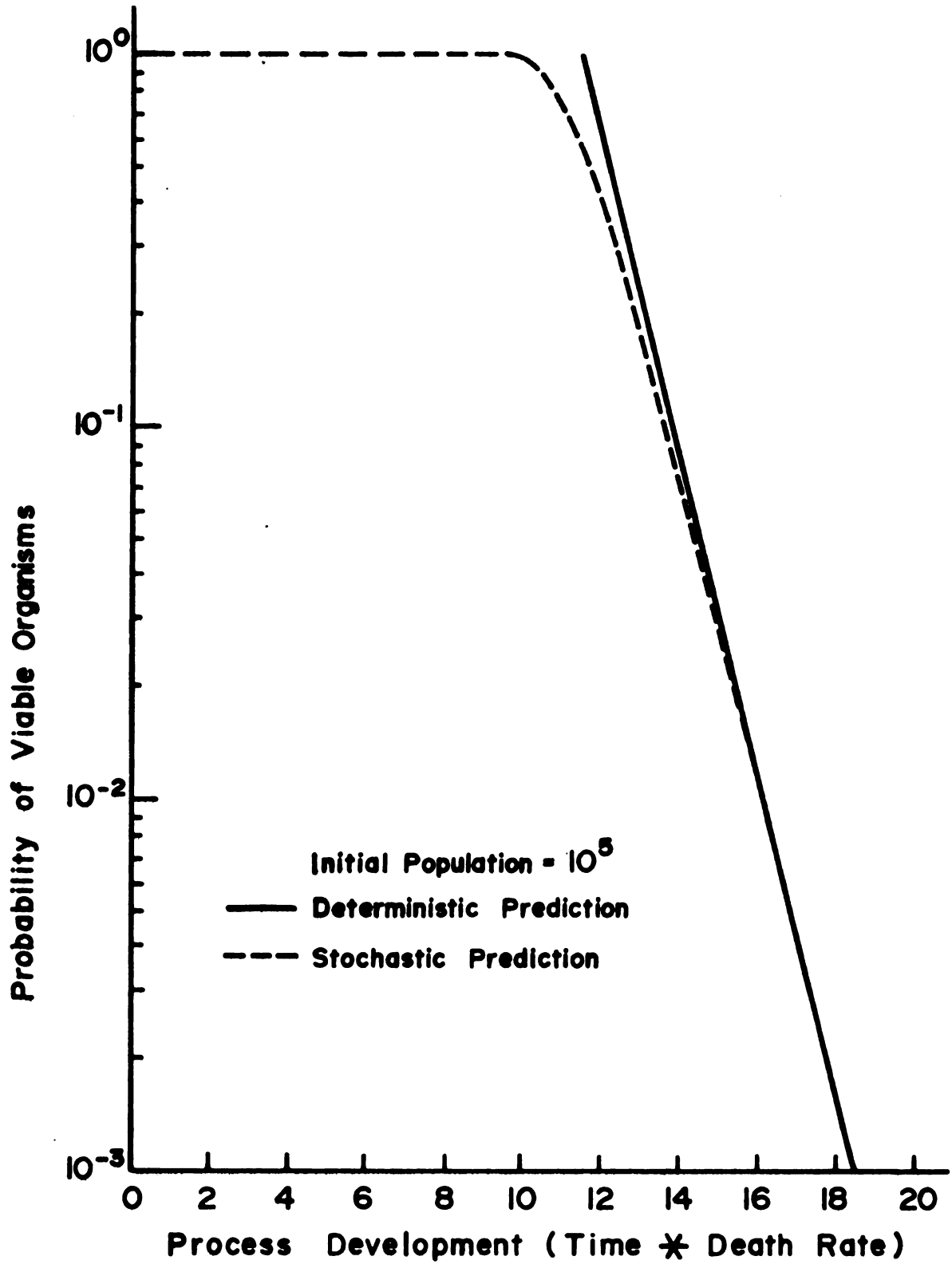

Figure 3.5.--Comparison of Deterministic and Stochastic Predictions of Viable Organisms Left in Population.

viability is down to .01, the difference between the two models is indistinguishable. Thus Equations 3.21 and 3.22 yield the same result for small q. This conclusion can also be procured by a Taylor series expansion of these two equations about the point q = 0. Since most processes designed to produce sterility would demand a probability of sterility of less than .01, the deterministic model is as good as the stochastic model for predicting the required process time. This last conclusion implies that the deterministic model is sufficient for sterility applications even though the stochastic model more accurately represents real homogeneous death processes.

Experimental Evidence

To find experimental evidence that stochastic models represent a death process better than deterministic models, the experimental variation from the mean was studied. Assuming a homogeneous process, the variation of the result from the mean can be a result of two factors. First, the intrinsic randomness of the process will cause variation. Second, errors of experimentation will also contribute to deviations from the expected population size. The latter factor can be caused by a large number of factors since microorganisms respond to many environmental factors. Hopefully, the researcher is able to control most of these variables. But this is a very difficult task. In all cases the part of the deviation from the mean caused by

experimental error and that part due to the true process variation can not be distinguished.

As already stated, the direct observation of microbial death events is not possible with today's technology. Therefore, death processes of large populations must be studied. Data of this type can be obtained by subjecting a large number of samples of an organism to a lethal condition and withdrawing samples at different time intervals and counting the number of organisms remaining.

Experimental data obtained by Dewey[1] working with Serratia marcescens were analyzed. He irradiated (X-rays) cells of this organism in an oxygen atmosphere. The results of these tests are shown in Table 3.1. Ordinary microbiological techniques were used in counting populations. This included the diluting of large populations to obtain a population small enough to count.

First, the death constant, h, was determined from a linear regression of the logarithm of the percent population reduction versus the dose of irradiation received for each trial. A death constant of .585 $(\text{kilorads})^{-1}$ was obtained. Using this value for h to determine the theoretical mean and standard deviation (Equations 3.2 and 3.3), the values in columns 5 and 7 of Table 3.1 were

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[1] See Dewey (1963) for a report of his experiments. The data reported here is not published in his article, but it was obtained through personal communication.

TABLE 3.1.--Analysis of irradiation (X-rays) of *Serratia marcescens*, experimental data of Dewey (1963).

Exp.	Initial Population	X-ray Dose Kilorads	Population[1] After Irradiation	Theoretical Mean	Population Deviation From Mean	Theoretical Standard Deviation
A	666,666,670	36.109	.33	.4467	.11	.6683
B	568,666,670	35.077	1.0	.6968	.30	.8347
C	1,290,000,000	32.661	4.33	4.4962	2.16	2.5488
D	323,000,000	27.558	23.0	32.1923	9.19	5.6738
E	29,300,000	27.297	5.5	3.4020	2.01	1.8444
F	173,733	13.880	16.67	51.7010	35.03	7.1893
G	86,866	10.410	112.33	196.8180	84.48	14.0133
H	86.866	4.940	1980.0	1498.5146	481.49	38.3753
I	86,866	3.470	15,363.33	11,409.2490	3954.08	99.5527
J	25,333	12.840	25.33	13.8526	11.48	3.7209
K	25,333	9.630	124.33	90.5884	33.74	9.5008
L	25,150	6.320	970.0	623.5402	346.46	24.6593
M	25,333	3.210	546.67	3873.9360	3327.27	57.2847
N	125,333	13.084	74.67	59.4170	15.23	7.7065
O	62,666	9.813	312.33	201.3370	111.00	14.1665
P	62,666	6.542	2246.67	1364.4613	882.21	36.5342
Q	62,666	3.271	14,903.33	9246.9584	5656.37	88.7834
R	125,066	12.980	90.0	63.0106	26.99	7.9359
S	62,533	9.735	294.67	210.2884	84.38	14.4769
T	62,533	6.490	2013.33	1403.6132	609.72	37.0419
U	62,533	3.245	15,293.33	9368.7038	5924.63	89.2473
V	108,000	12.420	152.33	75.5041	76.83	8.6863
W	54,000	9.315	312.67	232.1687	130.50	15.2043
X	54,000	6.210	1593.33	1427.7989	165.53	37.2833
Y	54,000	3.105	9686.67	8780.7250	905.94	85.7490
Z	60,066	12.836	35.67	32.9223	2.74	5.7362

AA	60,066	9.627	198.0	215.1672	17.17	14.6423
AB	60,066	6.414	1660.0	1409.5423	250.46	37.1007
AC	59,800	3.204	11,635.0	9176.6816	2458.32	88.1389
AD	88,866	13.428	64.33	34.4501	29.88	5.8683
AE	44,433	10.071	239.67	122.7574	116.91	11.0643
AF	44,433	6.714	1620.0	874.8520	745.15	29.2853
AG	44,433	3.357	9396.67	6234.7903	3161.88	73.2116
AH	40,966	12.828	58.0	22.5590	35.44	4.7483
AI	40,966	9.621	332.67	147.2463	185.40	12.1134
AJ	40,966	6.414	1820.0	961.3360	858.66	30.6395
AK	40,966	3.207	833.0	6275.5663	5442.23	72.8987
AL	46,900	14.220	5.67	11.4395	5.77	3.3818
AM	46,900	10.665	68.0	91.5374	23.54	9.5581
AN	46,900	7.110	806.67	732.4704	74.20	26.8520
AO	46,906	3.555	6946.67	5861.1315	1085.54	71.6146
AP	51,166	12.912	7.0	26.8247	19.82	5.1779
AQ	51,166	9.684	64.67	177.2752	112.61	13.2913
AR	51,166	6.456	770.0	1171.5507	401.55	33.8338
AS	51,166	3.228	7300.0	7742.3734	442.37	81.0606
AT	40,733	12.132	10.0	33.7028	23.70	5.8030
AU	40,733	9.099	92.67	198.7181	106.05	15.0623
AV	40,733	6.066	1010.0	1171.6778	161.66	33.7339
AW	40,733	3.033	11,500.0	6908.4255	4591.57	75.7413
AX	39,950	1.243	13.0	27.7666	14.77	5.2675
AY	40,766	9.322	161.0	174.5567	13.56	13.1837
AZ	39,950	6.215	1730.0	1053.2216	676.78	32.0227
BA	40,766	3.107	3866.67	6621.1545	2754.49	74.4699

[1]Fractional numbers are shown because an average of three samples was taken.

calculated. The difference between the theoretical mean and the population count is given in column 6.

If the deviation from the mean was only a result of the stochastic variation predicted, its expected value would be the theoretical standard deviation. A chi-square test could then be used to test the deviations from the mean against their expected values. This test was tried, but most of the experimental deviations were so much larger (as much as 50 times larger) than the theoretical ones that the test yielded a very negative result. However, this does not mean that the stochastic model was invalid because experimental errors could cause the deviations to be much larger than the theoretical prediction. Thus, the experimental errors would dwarf the effect of the intrinsic variation on the outcome. It is also possible that the intrinsic variation causes a larger deviation than predicted by the model.

By considering only the experiments for which the initial population was over one million, a good correlation was found between the theoretical and experimental deviations. Experiments A, B, C, D and E all had original populations over one million. They also had a population count of less than ten organisms after the irradiation treatment. A chi-square test for these five experiments showed the validity of the theoretical variance at a .05 level of significance.

An attempt was then made to correlate the experimental deviations to the theoretical standard deviations by a linear regression. A plot of these data is shown in Figure 3.6. From a least squares analysis of these data the following equation was obtained:

$$\text{Experimental Deviation} = 10.87 \text{ Theoretical Deviation} - 8.9 \qquad (3.23)$$

Thus, the experimental deviations were about eleven times that expected. These two variables had a correlation coefficient of .757. However, this correlation may be due to another variable such as the number of organisms remaining after the treatment. The dilution of the population required to obtain a countable number tends to amplify the errors for large populations. Also, the theoretical variance decreases as population decreases for the range of the experiments since all treatments extended beyond the dosage of maximum variance (Equation 3.4). Therefore, both may be correlated with population size.

In conclusion, the experimental results give evidence of the applicability of the theoretical model, but more accurate measurement techniques are needed to test the model.

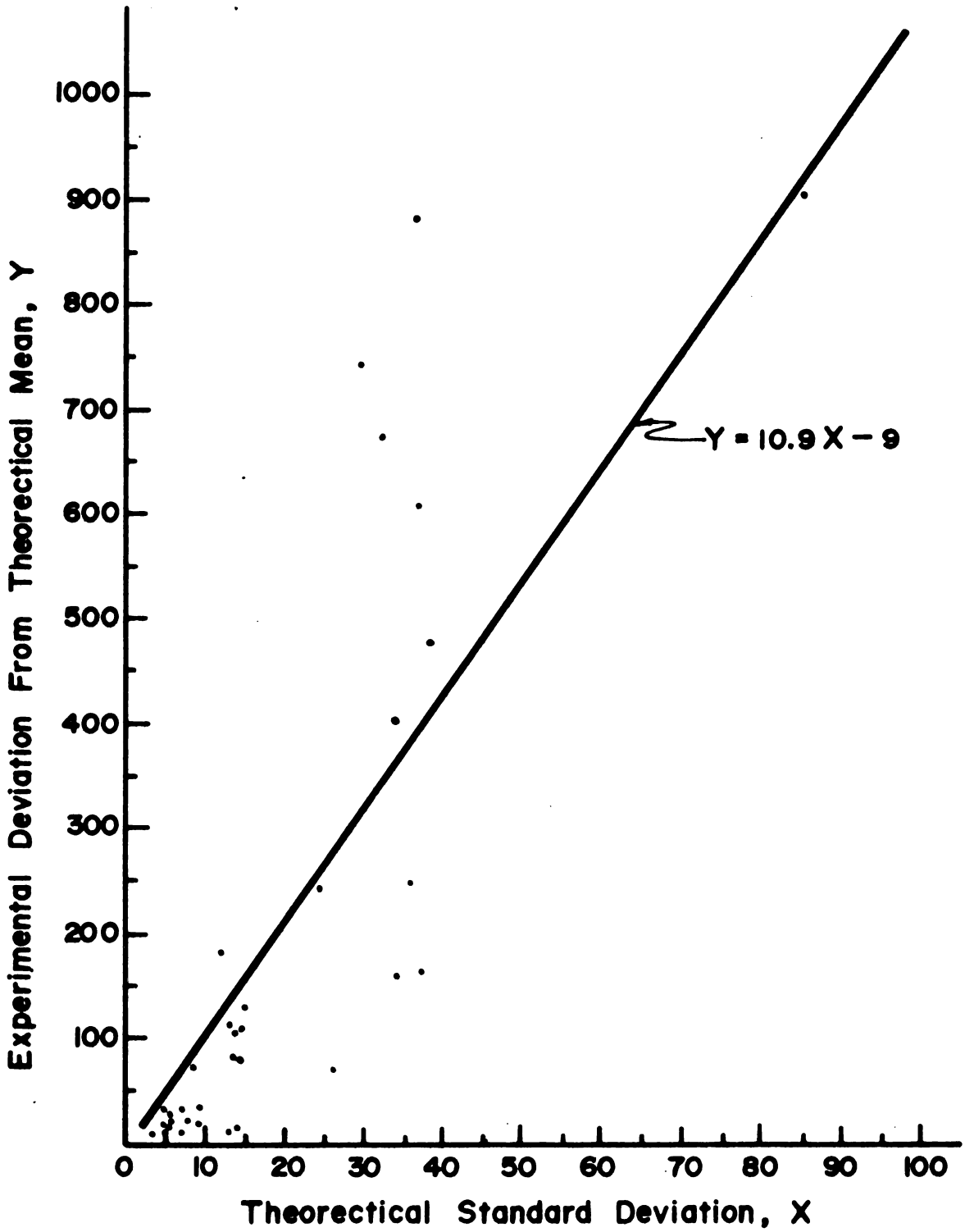

Figure 3.6.--Influence of Theoretical Standard Deviation on Experimental Deviation from Process Mean.

CHAPTER 4

A DEATH PROCESS WITH AN INTERMEDIATE STATE

In the elementary model of Chapter 2, there was only one possible transition: a change from a viable state to a non-viable state. As an extension of this model, one can theorize that the organism may exist in two different viable states. One state may be more death resistant than the other or one state may be a result of the lethal environment. The second viable state could also be a result of an adjustment to the lethal environment or a partially destroyed state.

To construct a stochastic model for this case, let the two viable states be denoted by 1 and 2, and the non-viable state as state 3. Assume organisms in states 1 and 2 both may become non-viable by transitions to state 3. In addition, assume organisms in state 1 may make a transition to state 2. All other possible transitions between the three states is assumed non-existent. Visually, this model could be represented by Figure 4.1.

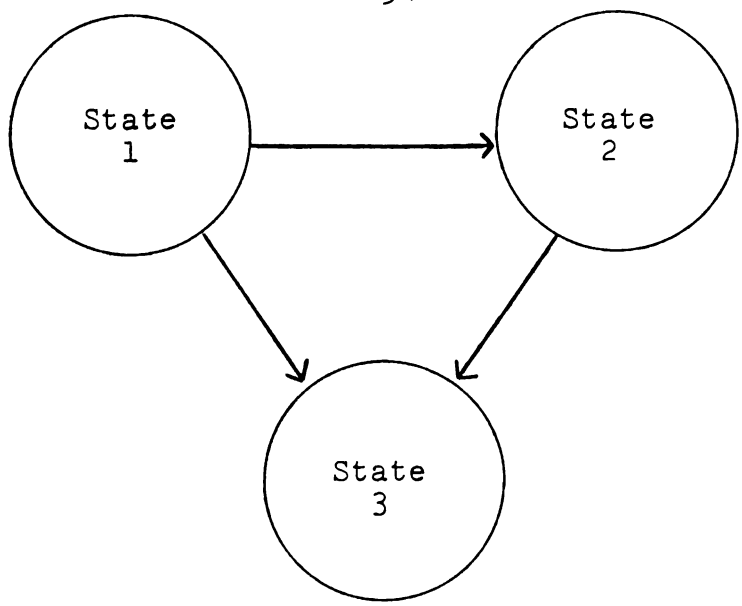

Figure 4.1.--Three State Model with Transitions Shown by Arrows.

To derive a stochastic model for the semi-closed time interval $0 \leq t < \infty$, let the number of organisms in each state be represented by the discrete random variables as follows:

$\ell(t)$ = number in state 1 at time t.

$m(t)$ = number in state 2 at time t.

$n(t)$ = number in state 3 at time t.

The joint probability distribution $p_{\ell,m,n}^{(t)}$ is defined as the probability of having ℓ organisms in state 1, m organisms in state 2 and n organisms in state 3 at time t. It follows that:

$$\sum_{\ell,m,n} p_{\ell,m,n}^{(t)} = 1 \qquad (4.1)$$

for all t $(0,\infty)$.

Further, assume that the initial concentration in each state is known; that is, $\ell(o) = L_o$, $m(o) = M_o$ and $n(o) = N_o$. Under these conditions,

$$n(t) = L_o + M_o + N_o - \ell(t) - m(t) \qquad (4.2)$$

Therefore, $p_{\ell,m,n}^{(t)}$ may be reduced to the two state probability $p_{\ell,m}^{(t)}$ since n(t) is known if ℓ and m are chosen. Equation 4.1 then reduces to:

$$\sum_{\ell,m} p_{\ell,m}^{(t)} = 1 \qquad (4.3)$$

The following system of 6 axioms is accepted to derive an analytical expression for $p_{\ell,m}^{(t)}$.

1. The probability of a transition of any organism in state 1 to state 2 during a short time interval t, t+Δt is represented by the transition parameter $h_1(t+\phi_1\Delta t)\Delta t$. The function ϕ_1 is chosen such that

$$\frac{1}{\Delta t}\int_t^{t+\Delta t} h_1(\tau)d\tau = h_1(t+\phi_1\Delta t). \qquad (4.4)$$

2. The probability of a transition of any organism in state 2 to state 3 during a short interval t, t+Δt is $h_2(t+\phi_2\Delta t)\Delta t$. And ϕ_2 is defined such that

$$\frac{1}{\Delta t}\int_t^{t+\ t} h_2(\tau)d\tau = h_2(t+\phi_2\Delta t). \qquad (4.5)$$

3. The probability of a transition of any organism in state 1 to state 3 during a short interval t, t+Δt is given by the transition parameter $g_1(t+\phi_3\Delta t)\Delta t$, where ϕ_3 is defined in the same way as ϕ_1 and ϕ_2 in Equations 4.4 and 4.5.
4. The probability of more than one transition among the three possible types in time interval t, t+Δt is o(Δt). Where o(Δt) is the zero order of Δt. That is, o(Δt) is defined such that

$$\lim_{\Delta t \to 0} \frac{o(\Delta t)}{\Delta t} = 0. \qquad (4.6)$$

5. All possible transitions other than given in axioms 1, 2, 3 and 4 have probability zero.
6. The joint occurrence of events occurring in non-overlapping time intervals is statistically independent.

To obtain an expression for $p_{\ell,m}^{(t+\Delta t)}$, consider the various transitions which, starting at time t, can lead to values ℓ and m for states 1 and 2 respectively at time t+Δt. Since the axioms listed above dictate that the random variables can either increase by one, decrease by one or remain the same without having a probability of order o(Δt), the transitions shown below in Table 4.1 must be considered.

TABLE 4.1.--Transitions for model.

Event	State At Time t	Transition During Δt	State At $t+\Delta t$
E_1	$\ell+1$, $m-1$	From state 1 to state 2	ℓ,m
E_2	ℓ, $m+1$	From state 2 to state 3	ℓ,m
E_3	$\ell+1$, m	From state 1 to state 3	ℓ,m
E_4	ℓ, m	No transitions	ℓ,m

The probability of the first three events listed in Table 4.1 above is the product of the probability of two independent events. The first event is the occurrence of being in the state specified at time t. Its probability is given by definition. The second independent event has the probability of a transition during time Δt. Axioms 1, 2 and 3 specify these probabilities. Consequently,

$$p\{E_1\} = p^{(t)}_{\ell+1,m-1} (\ell+1)\, h_1(t+\phi_1\Delta t)\Delta t \qquad (4.7)$$

$$p\{E_2\} = p^{(t)}_{\ell,m+1} (m+1)\, h_2(t+\phi_2\Delta t)\Delta t \qquad (4.8)$$

$$p\{E_3\} = p_{\ell+1,m} (\ell+1)\, g_1(t+\phi_3\Delta t)\Delta t. \qquad (4.9)$$

If more than one event of any of the three types given above or any combination of them occurs, then $o(\Delta t)$ must be a factor in the probability according to axiom 4.

Let E_4 be the event that the process is in state ℓ,m after two or more transitions during time interval Δt. If r and s are any combination such that after two or more transitions state r,s becomes state ℓ,m,

$$p\{E_4\} = \sum_{r,s} p_{r,s}(t)\ o(\Delta t) \tag{4.10}$$

Event 5 can now be defined as occurring if no transitions take place during interval Δt. Because all possible events must have a total probability of one, the probability of event 5 is one less all probabilities of one or more transitions. Therefore,

$$p\{E_5\} = p_{\ell,m}(t)\ (1 - q) \tag{4.11}$$

where

$$q = \ell h_1(t+\phi_1\Delta t)\Delta t + m h_2(t+\phi_2\Delta t)\Delta t$$

$$+ \ell g_1(t+\Delta t)\Delta t + o(\Delta t). \tag{4.12}$$

Since the five events are mutually exclusive ways in which $p_n(t+\Delta t)$ may occur, the desired probability is the summation of the probabilities for each of the five events.

$$p_{\ell,m}(t+\Delta t) = p\{E_1\} + p\{E_2\} + p\{E_3\} + p\{E_4\} + p\{E_5\} \tag{4.13}$$

$$p_{\ell,m}(t+\Delta t) = p^{(t)}_{\ell+1,m-1}\,(\ell+1)h_1(t+\phi_1\Delta t)\Delta t$$

$$+ p^{(t)}_{\ell,m+1}\,(m+1)h_2(t+\phi_2\Delta t)\Delta t$$

$$+ p^{(t)}_{\ell+1,m}\,(\ell+1)g_1(t+\phi_3\Delta t)\Delta t$$

$$+ p^{(t)}_{\ell,m}\Big[1 - \ell h_1(t+\phi_1\Delta t)\Delta t - m h_2(t+\phi_2\Delta t)\Delta t$$

$$- \ell g_1(t+\phi_3\Delta t)\Delta t - o(\Delta t)\Big] + \sum_{r,s} p_{r,s}(t)o(\Delta t)$$

(4.14)

This equation can then be reduced to a differential equation by subtracting $p_{\ell,m}(t)$ from both sides, dividing by Δt and taking limit as Δt approaches zero. This equation is:

$$\frac{d\,p_{\ell,m}(t)}{dt} = p^{(t)}_{\ell+1,m-1}\,(\ell+1)h_1(t) + p^{(t)}_{\ell,m+1}\,(m+1)h_2(t)$$

$$+ p^{(t)}_{\ell+1,m}\,(\ell+1)g_1(t) - p_{\ell,m}(t)\Big[\ell\Big(h_1(t)$$

$$+g_1(t)\Big) + m h_2(t)\Big] \qquad (4.15)$$

Taking into account that $p_{\ell,m}(t)$ has value zero for $\ell > L_o$, $m > M_o$ and ℓ or m less than zero, Equation 4.15

may be reduced to a simpler form for the end points of the process.

$$\frac{d\, P_{L_o,M_o}(t)}{dt} = - p_{L_o,M_o}(t)\left[\ell\left[h_1(t) + g_1(t)\right] + mh_2(t)\right] \tag{4.16}$$

$$\frac{d\, p_{0,0}(t)}{dt} = p_{1,0}(t)g_1(t) + p_{0,1}h_2(t) \tag{4.17}$$

As indicated in Chapter 2, the easiest method of solving the system of equations in 4.15 is by using a generating function. Therefore, the following bivariate probability generating function is defined.

$$\psi(x,y,t) = \sum_{\ell=0}^{L_o} \sum_{m=0}^{M_o} p_{\ell,m}(t)\, x^{\ell} y^{m} \tag{4.18}$$

From this definition,

$$\frac{\partial \psi}{\partial t} = \sum_{\ell,m} x^{\ell} y^{m} \frac{d\, p_{\ell,m}(t)}{dt} \tag{4.19}$$

$$\frac{\partial \psi}{\partial x} = \sum_{\ell,m} \ell x^{\ell-1} y^{m}\, p_{\ell,m}(t) \tag{4.20}$$

$$\frac{\partial \psi}{\partial y} = \sum_{\ell,m} m\, x^{\ell} y^{m-1}\, p_{\ell,m}(t) \tag{4.21}$$

Multiplying each term in Equation 4.15 by $x^{\ell}y^{m}$ and summing over all possible values of ℓ and m, Equation 4.15 becomes

$$\sum_{\ell,m} x^{\ell}y^{m} \frac{d\, p_{\ell,m}(t)}{dt} = \sum_{\ell,m} (\ell+1)x^{\ell}y^{m}h_1(t)\, p_{\ell+1,m-1}$$

$$+ \sum_{\ell,m} (m+1)x^{\ell}y^{m}h_2(t)\, p_{\ell,m+1}$$

$$+ \sum_{\ell,m} (\ell+1)x^{\ell}y^{m}g_1(t)\, p_{\ell+1,m}$$

$$- \sum_{\ell,m} \ell x^{\ell}y^{m}\Big(h_1(t) + g_1(t)\Big)\, p_{\ell,m}(t)$$

$$- \sum_{\ell,m} m x^{\ell}y^{m}h_2(t)\, p_{\ell,m}(t) \qquad (4.22)$$

The above can be reduced to a single partial differential equation by substituting the functions defined in Equations 4.18 through 4.21 along with a few shifts of axes.

$$\frac{\partial \psi(x,y,t)}{\partial t} = yh_1(t)\, \frac{\partial \psi}{\partial x} + h_2(t)\, \frac{\partial \psi}{\partial y} + g_1(t)\, \frac{\partial \psi}{\partial x}$$

$$- x\Big(h_1(t) + g_1(t)\Big)\, \frac{\partial \psi}{\partial x} - yh_2(t)\, \frac{\partial \psi}{\partial y}$$

(4.23)

This may be reduced to

$$0 = \left[yh_1(t) + g_1(t) - x\left(h_1(t) + g_1(t)\right)\right] \frac{\partial \psi}{\partial x}$$

$$+ h_2(t)(1 - y) \frac{\partial \psi}{\partial y} - \frac{\partial \psi}{\partial t} \qquad (4.24)$$

Using LaGrange's method of auxiliary ordinary differential equations (Appendix B), Equation 4.24 has the same solution as:

$$\frac{d\psi}{0} = \frac{dx}{yh_1(t) + g_1(t) - \left(h_1(t) + g_1(t)\right)x}$$

$$= \frac{dy}{h_2(t)(1 - y)} = \frac{dt}{-1} \qquad (4.25)$$

The solution for this was obtained for initial conditions that there were L_o in state 1 and M_o in state 2 at the beginning of the process. The complete derivation is given in Appendix C. The solution is:

$$\psi(x,y,t) = \left[x\beta + y\gamma + 1 - \beta - \gamma\right]^{L_o} \left[y\alpha + 1 - \alpha\right]^{M_o} \qquad (4.46)$$

where

$$\alpha = \exp\left(- \int_o^t h_2(\tau)d\tau\right) \qquad (4.27)$$

$$\beta = \exp\left(- \int_o^t \left[h_1(\tau) + g_1(\tau)\right]d\tau\right) \qquad (4.28)$$

$$\gamma = \alpha \int_o^t h_1(\tau)\exp\left(- \int_o^t \left[h_1(\tau) + g_1(\tau) - h_2(\tau)\right]d\tau\right)d\tau \qquad (4.29)$$

The joint probability distribution for ℓ in state 1 and m in state 2 is found by selecting the coefficients of x^{ℓ} and y^{m} in Equation 4.26. By series expansion,

$$p_{\ell,m}(t) = \binom{L_o}{\ell}\beta^{\ell}\sum_{i=0}^{m}\binom{L_o-\ell}{i}\gamma^{i}\left[1-\beta-\gamma\right]^{L_o-\ell-i}\binom{M_o}{m-i}\alpha^{m-i}(1-\alpha)^{M_o-m+i} \tag{4.30}$$

The summation in the above equation includes all terms from zero to m, but only the terms where $\binom{L_o-\ell}{i}$ and $\binom{M_o}{m-i}$ are defined need be included. All others have value zero.

If M_o is zero,

$$p_{\ell,m}(t) = \binom{L_o}{\ell}\beta^{\ell}\binom{L_o-\ell}{m}\gamma^{m}\left[1-\beta-\gamma\right]^{L_o-\ell-m} \tag{4.31}$$

If L_o is zero, the joint distribution is not required because ℓ will start at zero and remain there for the whole process. In this case the stochastic model reduces to the elementary model presented in Chapter 2.

According to Equation 4.30, the probability of extinction is given by

$$p_{0,0}(t) = \left[1-\beta-\gamma\right]^{L_o}\left[1-\alpha\right]^{M_o} \tag{4.32}$$

From the definition of the bivariate generating function given in Equation 4.18, the generating functions of the marginal distributions for state 1 and state 2 , $\psi_1(x,t)$ and $\psi_2(y,t)$ respectively, may be specified.

$$\psi_1(x,t) = \psi(x,1,t) = [x\beta + 1 - \beta]^{L_o} \tag{4.33}$$

$$\psi_2(y,t) = \psi(1,y,t) = [y\alpha + 1 - \alpha]^{M_o}[y\gamma + 1 - \gamma]^{L_o} \tag{4.34}$$

Using Equation 4.33 to determine the probability distribution for the number of organisms in state 1, it yields the binomial distribution:

$$p_\ell(t) = \binom{L_o}{\ell}\beta^\ell(1 - \beta)^{L_o-\ell} \tag{4.35}$$

with mean

$$\bar{\ell}(t) = L_o\beta \tag{4.36}$$

and variance

$$\sigma_\ell^{\,2}(t) = L_o\beta(1 - \beta) \ . \tag{4.37}$$

For state 2, the distribution $p_m(t)$, mean $\bar{m}(t)$ and variance $\sigma_2^{\,2}(t)$ are derived from Equation 4.34. It follows that:

$$p_m(t) = \sum_{i=0}^{m} \binom{L_o}{i} \gamma^i (1-\gamma)^{L_o-i} \binom{M_o}{m-i} \alpha^{m-i} (1-\alpha)^{M_o-m+i} \tag{4.38}$$

$$\bar{m}(t) = M_o\alpha + L_o\gamma \tag{4.39}$$

$$\sigma_m^2(t) = M_o\alpha(1-\alpha) + L_o\gamma(1-\gamma). \tag{4.40}$$

The generating function for the joint distribution is also used to obtain the covariance (cov) of states 1 and 2. Since

$$\mathrm{cov}(\ell,m) = E(\ell,m) - E(\ell)\,E(m) \tag{4.41}$$

where E denotes the expected value and ℓ and m are the usual time dependent variables. The expected values of ℓ and m are given in Equations 4.36 and 4.39, respectively. And

$$E(\ell,m) = \left.\frac{\partial^2 \psi(x,y,t)}{\partial x \partial y}\right|_{x=y=1} \tag{4.42}$$

Making the prescribed substitutions,

$$\mathrm{cov}(\ell,m) = -L_o\beta\gamma \tag{4.43}$$

In addition the probability distribution for the total number in states 1 and 2 may easily be obtained. The probability generating function $\psi_k(z,t)$ for this distribution is again acquired from the joint distribution generating function as given by the following equation from Feller (1957, p. 261).

$$\psi_k(z,t) = \Psi(z,z,t) \tag{4.44}$$

therefore,

$$\psi_k(z,t) = \left[z(\beta+\gamma) + 1 - (\beta+\gamma)\right]^{L_o} \left[z\alpha + 1 - \alpha\right]^{M_o} \tag{4.45}$$

If k(t) indicates the random variable characterizing the total number in states 1 and 2,

$$p_k(t) = \sum_{i=0}^{k} \binom{L_o}{i} (\beta+\gamma)^i (1-(\beta+\gamma))^{L_o-i} \binom{M_o}{k-i} \gamma^{k-i} (1-\gamma)^{M_o-k+i} \tag{4.46}$$

and

$$\bar{k}(t) = L_o(\beta+\gamma) + M_o\alpha \tag{4.47}$$

$$\sigma_k^2(t) = L_o(\beta+\gamma)(1-\beta-\gamma) + M_o\alpha(1-\alpha) \tag{4.48}$$

Using Equation 4.46 to obtain the probability of extinction, the result is the same as given in 4.31; i.e.,

$$p_o(t) = [1-\beta-\gamma]^{L_o} [1-\gamma]^{M_o} \tag{4.49}$$

CHAPTER 5

HOMOGENEOUS CASE OF MODEL WITH AN INTERMEDIATE STATE

Description

If the transition parameters $h_1(t)$, $h_2(t)$ and $g_1(t)$ of the model described in Chapter 4 are independent of time, the homogeneous case results. The model could be visually represented by Figure 5.1. As defined in Chapter 4, $\ell(t)$ and $m(t)$ are the number in states 1 and 2 respectively, and $\ell(o) = L_o$, $m(o) = M_o$. Also the probability generating $\psi(x,y,t)$ is defined as $\sum_{\ell,m} p_{\ell,m}(t)x^{\ell}y^{m}$.

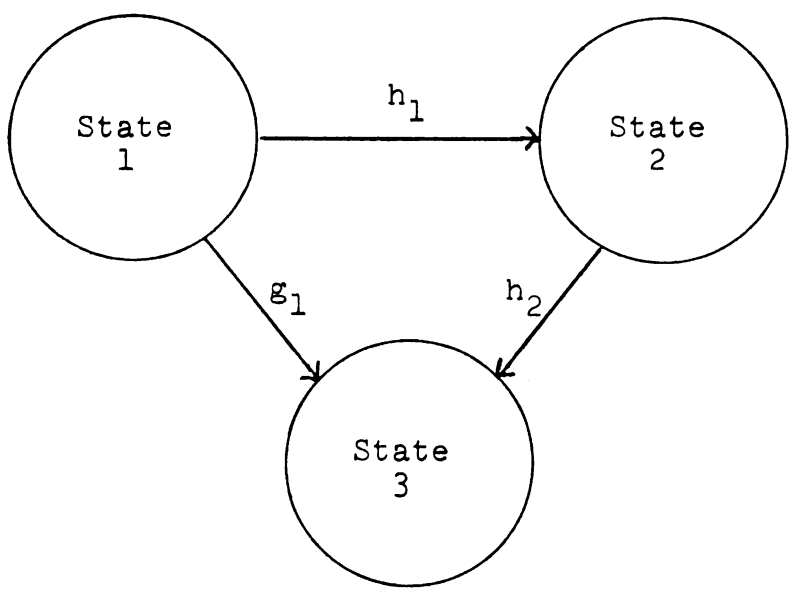

Figure 5.1.--Three State Model with Constant Transition Parameters h_1, h_2 and g_1.

Replacing $h_1(t)$, $h_2(t)$ and $g_1(t)$ by the constants h_1, h_2 and g_1 respectively in Equation 4.26 through 4.29,

$$\Psi(x,y,t) = [xb + yc + 1 - b - c]^{L_o} [ya + 1 - a]^{M_o} \tag{5.1}$$

where

$$a = \exp(-h_2 t) \tag{5.2}$$

$$b = \exp(-(h_1+g_1)t) \tag{5.3}$$

$$c = \frac{h_1}{h_1+g_1-h_2} \left(\exp(-h_2 t)-\exp(-(h_1+g_1)t)\right) \tag{5.4}$$

If $h_1 + g_1 = h_2$, c can not be defined by Equation 5.4. In this special case

$$c = h_1 t \exp(-h_2 t) \tag{5.5}$$

To obtain Equation 5.5, either the limit $h_2 \to h_1 + g_1$ of Equation 5.4 may be derived, or the original Equation (4.29) used to derive Equation 5.4 may be evaluated for this particular case.

By substituting the expressions given for a, b and c (Equations 5.2 through 5.4) for α, β, and γ, respectively, in the equations of Chapter 4, all the results of the non-homogeneous model may be evaluated for the homogeneous model of this chapter. Several applications of these developments will now be considered.

An Example of Heat Kinetics

Shull, Cargo and Ernst (1963) proposed a deterministic model of the type given in this chapter except they assumed the rate of transition from state 1 to state 3 to be zero. Their model was intended to represent the kinetics of heat activation and thermal death of bacterial spores. At the start of a thermal death process, some bacteria were considered to first undergo a heat activation process and then a death process due to the hot environment. Other bacteria were considered to be in a state where germination was possible without heat activation. Shull et al. (1963) used this model to describe the death curves of Bacillus stearothermophilus spores.

In terms of the three states of Figure 5.1, state 1 would represent the organisms that require heat activation to germinate. The activation process would then be represented by a transition to state 2. All organisms which would reproduce without heat activation would be considered to be in state 2 at the start of the process. From this state, the organisms would become non-viable by a transition to state 3.

Frederickson (1966b) extended the model proposed by Shull et al. (1963) by including the possibility of a direct transition from the unactivated condition (state 1) to the non-viable condition (state 3). He suggests that a stochastic model should be used to represent the process,

but he only gives the derivation of the deterministic equations or the mean of the stochastic model. The complete probability distribution for this model can be obtained from the probability generating function given in 5.1.

Since the organisms in state 1 would require heat activation to become countable by standard counting techniques, the number in state 2 is of particular interest. Its generating function $\psi_2(y,t)$ can easily be obtained from Equation 4.34. Accordingly

$$\psi_2(y,t) = [yc + 1 - c]^{L_o} [ya + 1 - a]^{M_o} \qquad (5.6)$$

And using Equations 4.38 through 4.40, the probability distribution $p_m(t)$, mean $\bar{m}(t)$, and variance $\sigma_m^2(t)$ are as follows:

$$p_m(t) = \sum_{i=0}^{m} \binom{L_o}{i} c^i (1-c)^{L_o-i} \binom{M_o}{m-i} a^{m-i} (1-a)^{M_o-m+i} \qquad (5.7)$$

$$\bar{m}(t) = M_o a + L_o c \qquad (5.8)$$

$$\sigma_m^2(t) = M_o a(1-a) + L_o c(1-c) \qquad (5.9)$$

The mean would increase at the start of the process in most examples, then die away to zero. To determine the cases where the mean is maximum at the start of the process, consider the time the derivative is zero.

Letting t_m be the time the mean is a maximum,

$$t_m = \frac{1}{h_1+g_1-h_2} \ln \left[\frac{L_o h_1 (h_1+g_1)}{h_2 M_o (h_1+g_1-h_2) + h_2 L_o h_1} \right] \qquad (5.10)$$

$$\text{where } h_1+g_1 \neq h_2$$

The maximum will be at t = 0 if Equation 5.10 yields a non-positive value.

If $h_1+g_1 = h_2$,

$$t_m = \frac{1}{h_2} \left[1 - \left(\frac{M_o h_2}{L_o h_1} \right) \right] \qquad (5.11)$$

$$= 0 \quad \text{if } M_o h_2 \geq L_o h_1$$

In addition, the time the variance is maximum will occur between the end points of the time interval $(0,\infty)$ because $\sigma_m^2(o) = \sigma_m^2(\infty) = 0$ according to Equation 5.9. The time of maximum variance is the solution to the equation

$$\left(\frac{2c-1}{2a-1} \right) \left[(h_1+g_1)\exp(-(h_1+g_1-h_2)t) - h_2 \right] = \frac{M_o h_2 (h_1+g_1-h_2)}{L_o h_1}$$

$$\text{where} \quad h_1+g_1 \neq h_2 \qquad (5.12)$$

and

$$\left(\frac{1 - 2h_1 t\ a}{1 - 2a}\right)\left(1 - h_2 t\right) = \frac{h_2\ M_o}{h_1\ L_o} \qquad (5.13)$$

$$\text{if} \quad h_1 + g_1 = h_2 \ .$$

Since these equations are both implicit equations, it is impossible to make any general observation about the size of the maximum variance and the time of the maximum variance in relation to the time of the maximum value for the mean.

This process may be simulated in a manner similar to that used for the simple death process in Chapter 3. Using a computer with a library function to generate random numbers with a uniform distribution over the interval (0,1), a step-by-step process can be simulated in the following manner. For ℓ in state 1 and m in state 2, the axioms of Chapter 4 may be written for the homogeneous model. The three possible transitions have the following probabilities for a very small time interval Δt

$$p\{\text{transition from state 1 to state 3}\} = \Delta p_1 = \ell g_1 \Delta t \qquad (5.14)$$

$$p\{\quad " \quad " \quad " \quad 1 \text{ to } " \quad 2\} = \Delta p_2 = \ell h_1 \Delta t \qquad (5.15)$$

$$p\{\quad " \quad " \quad " \quad 2 \text{ to } " \quad 3\} = \Delta p_3 = m h_2 \Delta t \qquad (5.16)$$

If Δt is sufficiently small, the probability of more than one of these events occurring during Δt will be insignificant. To insure this condition, a Δt was picked such that the maximum sum of Δp_1, Δp_2 and Δp_3 was less than .1. A computer program was written to perform the following steps:

1. Initialize the program for the number in state 1 and state 2 at time zero.
2. Calculate the probabilities of the possible transitions according to Equations 5.14, 5.15 and 5.16. Assign each of these probabilities to a different portion of the complete interval between 0 and 1 where the length of each portion is determined by the size of Δp_1, Δp_2 and Δp_3.
3. Generate a random number RN with a uniform distribution (0,1).
4. Increase time by Δt.
5. Check if RN falls within any of the intervals assigned in step 2. If it does not, repeat the above steps starting at the third one. If RN is in the interval determined by Δp_1, go to step 6. If RN is in the interval Δp_2, go to step 7; and if RN is in the interval Δp_3, go to step 8.
6. Decrease the number in state 1 by one and repeat the process starting at step 2.

7. Decrease the number in state 1 by one and increase the number in state 2 by one. Record the time and number in state 2. Then repeat the procedure starting at step 2.
8. Decrease the number in state 2 by one. Record the time and number in state 2. And repeat the process starting at step 2 until the number in both one and two are zero.

An example of this simulation procedure is shown in Figure 5.2.

This process can also be simulated by generating the time intervals between events in a manner similar to the one used in Chapter 3 (p. 36). Yet this procedure is complicated by the possibility of two types of transitions for both state 1 and state 2. For state 1 Equation 3.16 can be used to generate the time u when the number is decreased by one. So

$$u = \frac{-\log_e(RN)}{(h_1+g_1)\ell} \qquad (5.17)$$

where RN is a random number uniformly distributed over the interval (0,1). To distinguish if the reduction is caused by a transition from state 1 to state 3 or a transition from state 1 to state 2, a random experiment with these two outcomes could be performed with the probabilities proportional to the parameters of transition, g_1 and h_1. This procedure will simulate two transitions.

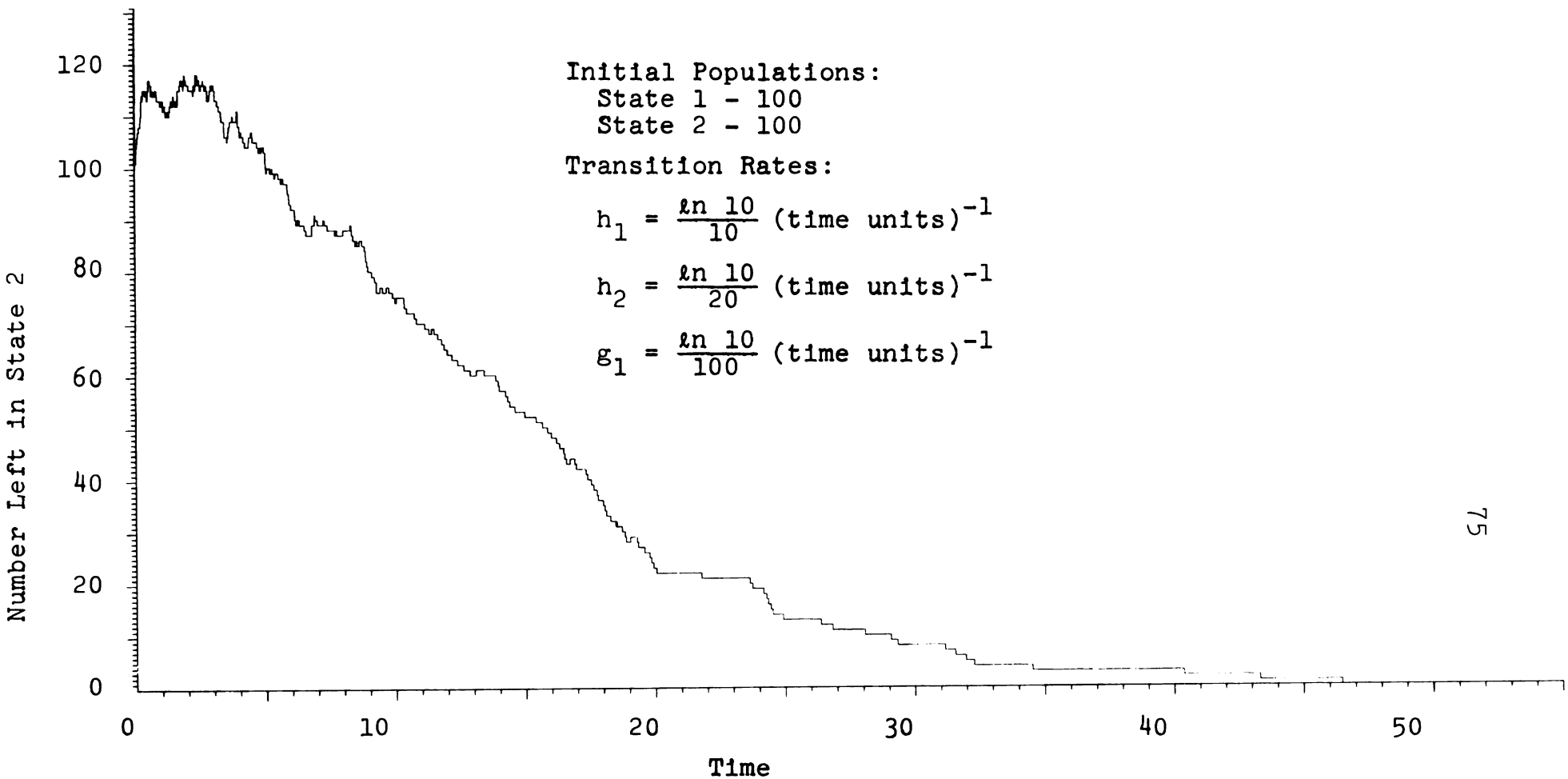

Figure 5.2.--Simulated Population (State 2) for Model with Two Viable States--Method Using Equations 5.14-16.

The only event left to generate is the time interval v between the transitions from state 2 to state 3. Because this transition has the same characteristics as the one in Chapter 3 used to derive Equation 3.15 which gives the time interval between events, Equation 3.15 may be used to find v. Therefore,

$$v = \frac{-\log_e(RN)}{h_2 m} \tag{5.18}$$

where RN is defined as before.

This simulation procedure will have an error because a transition to state 2 is not taken into account in determining the next transition from state 2 to state 3 until the next transition of the latter type occurs. This error will be insignificant if the reduction to zero of the number in state 1 is somewhat sooner than for state 2.

By using the above method, the complex form of the distribution for the number in state 2 (Equation 5.7) is avoided. However, the distribution of the time interval between a transition from state 1 to state 2 or from state 2 to state 3 involves a very simple form of the joint probability distribution given by Equation 4.30. But this approach would also incur errors of the type mentioned in the proceding paragraph.

A simulation was achieved with a computer program as follows:

1. Initialize the program for time zero. Generate the time of the first transition from state 1 by Equation 5.17 and the time of the first transition from state 2 to state 3 by Equation 5.18.
2. Compare the time of the next transition from state 1 to the time for the next transition from state 2. If the latter type occurs first, proceed to step 6; otherwise continue to step 3.
3. Generate a random number uniformly distributed on the interval (0,1). If this is less than the value of $h_1/(h_1+g_1)$, proceed to step 5; otherwise go to step 4. When ℓ reaches zero, the time of the next transition from state 1 should be set at a very large number so as to avoid any more transitions of this type.
4. Decrease ℓ by one and generate the time of the next transition from state 1 by adding the interval u (obtained by another evaluation of Equation 5.17) to the current time in the simulated process. Then return to step 2.
5. Decrease ℓ by one and increase m by one; record the time and size of m. Again obtain a new value of the interval u from Equation 5.17 and determine the time of the next transition of this type as in step 4. Then return to step 2.

6. Decrease m by one, record time and size of m. Generate a new value for v (Equation 5.18) and determine the time of the next transition from state 2 to state 3 by using this value. Continue this procedure until the number in state 2 reaches zero.

An example of the results of this type of simulation is shown in Figure 5.3.

To simulate laboratory data for this model, the method discussed in Chapter 3 (p. 38) may be utilized. The individual data points were generated by assuming a normal distribution as an approximation of a binomial distribution. Because the generating function (Equation 5.6) is the product of two binomial generating functions, the distribution (Equation 5.7) is the sum of several binomial factors. Consequently, a normal approximation can also be used to approximate the distribution. A random data point $m_r(t)$ can then be generated by the following equation

$$m_r(t) = \bar{m}(t) + V^*\sigma_m(t) \qquad (5.19)$$

where $\bar{m}(t)$ and $\sigma_m(t)$ are the mean and standard derivation defined in Equations 5.8 and 5.9. The variable V is a normal deviate which can be generated by Equation 3.10. Again the approximation of the distribution by a normal will be without significant error if the variance is sizable.

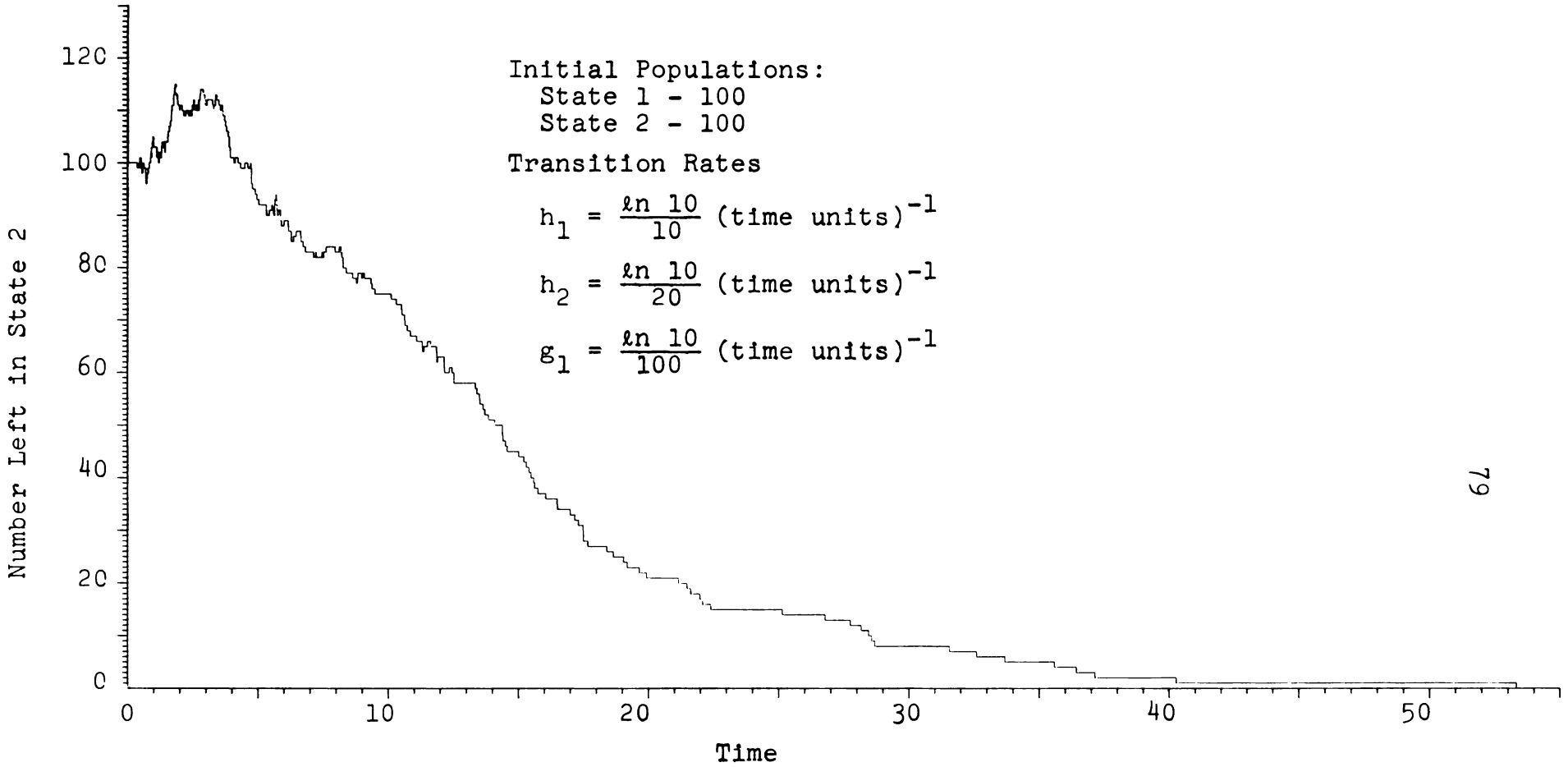

Figure 5.3.--Simulated Population (State 2) for Model with Two Viable States--Method of Generating Time Intervals Between Each Transition.

An Example of Change of Death Rate

The three state model of Chapter 4 is also proposed by Terui (1966) and used by Komenushi, Takada and Terui (1966) to represent a change of the death rate constant of Bacillus pumilus spores during heat sterilization. These researchers assumed that a spore may exist in two viable states, both which will germinate using standard culturing techniques. They theorized that spores in one state could make a transition to a state of lower or higher resistance to heat sterilization. The change is considered to be a discrete molecular change. Thus the viable organism can exist in only one state or another; there is no continuum between the possible states of existance. Prior to the work of Terui, Komemushi et al. (1966), Scharer and Humphrey (1963) had proposed a similar model to account for the non-logarithmic order of death curves for Bacillus stearothermophilus. But they gave only two possible transitions: one transition from an initial viable state to a second viable state and a transition from the second viable state to a non-viable state. On the other hand, Terui (1966) included direct transitions from both viable states to the non-viable state. Neither Terui nor Scharer suggests probabilistic models, but they give the deterministic equations.

The stochastic model for this example would be described by the generating function given in Equation

4.45. Adopting this generating function, $\psi_k(z,t)$, for the homogeneous case,

$$\psi_k(z,t) = [z(b+c) + 1 - b - c]^{L_o} [za + 1 - a]^{M_o} \quad (5.20)$$

This model gives the distribution for the total number in states 1 and 2 or $k = \ell + m$. As derived in Equations 4.46, 4.47 and 4.48, the probability distribution of k is the following

$$p_k(t) = \sum_{i=0}^{k} \binom{L_o}{i} (b+c)^i (1-b-c)^{L_o-i} \binom{M_o}{k-i} c^{k-i} (1-c)^{M_o-k+i} \quad (5.21)$$

with mean $\bar{k}(t)$ and variance $\sigma_k^2(t)$ given by

$$\bar{k}(t) = L_o(b+c) + M_o a \quad (5.22)$$

$$\sigma_k^2(t) = L_o(b+c)(1-b-c) + M_o a(1-a) \quad (5.23)$$

The values of a, b and c are the same as given at the beginning of the chapter in Equations 5.2 through 5.5.

The simulation of this process can be accomplished with the same computer programs presented in the previous sections. The only change required in these programs is the recording of the total number in states 1 and 2 instead of just the number in state 2. Figures 5.4 and 5.5 give examples of this type of simulation. The method to simulate time intervals between transitions (p. 73) was

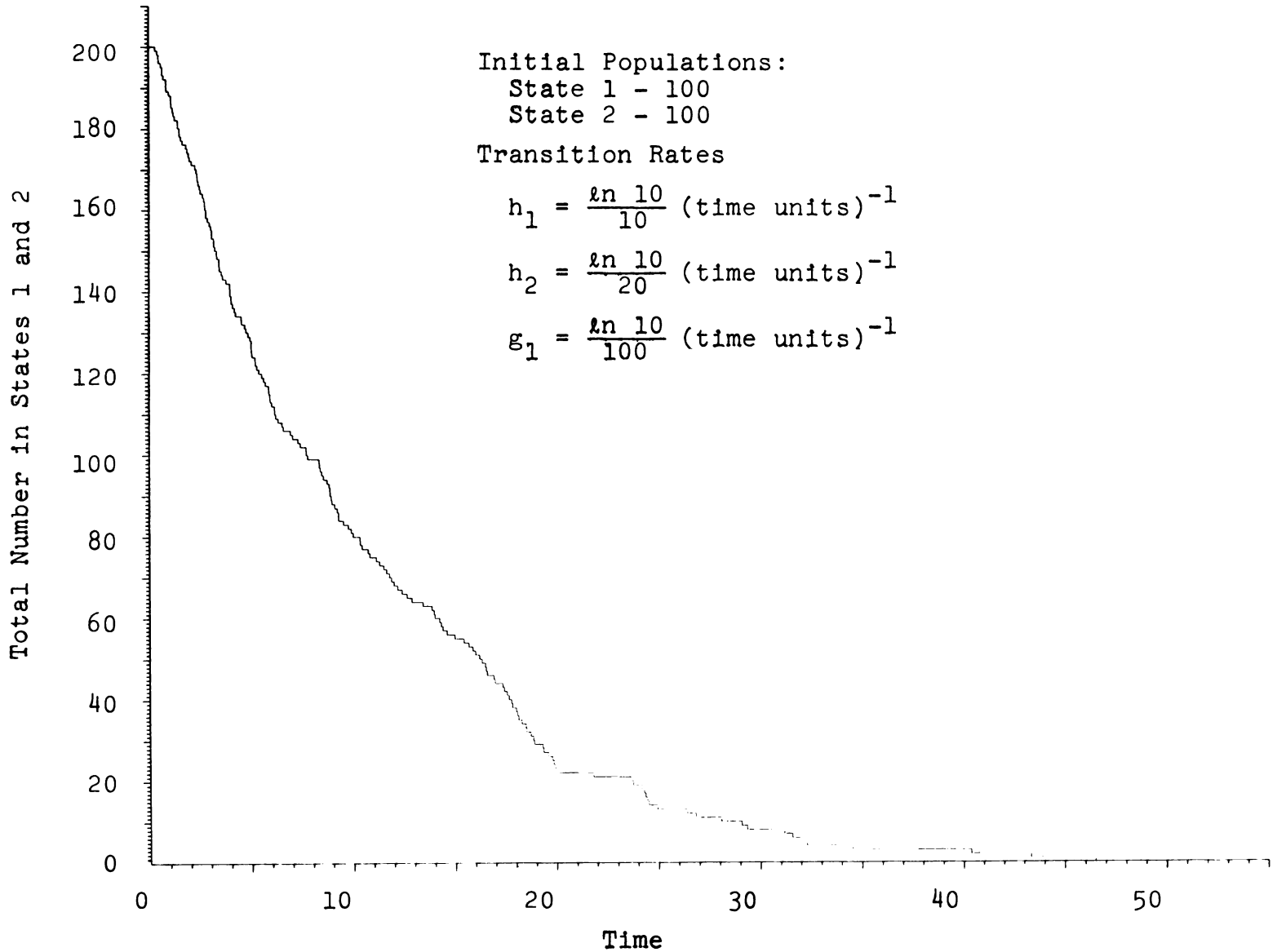

Figure 5.4.--Simulated Death Process for Model With Two Viable States--Method Using Equations 5.14-16.

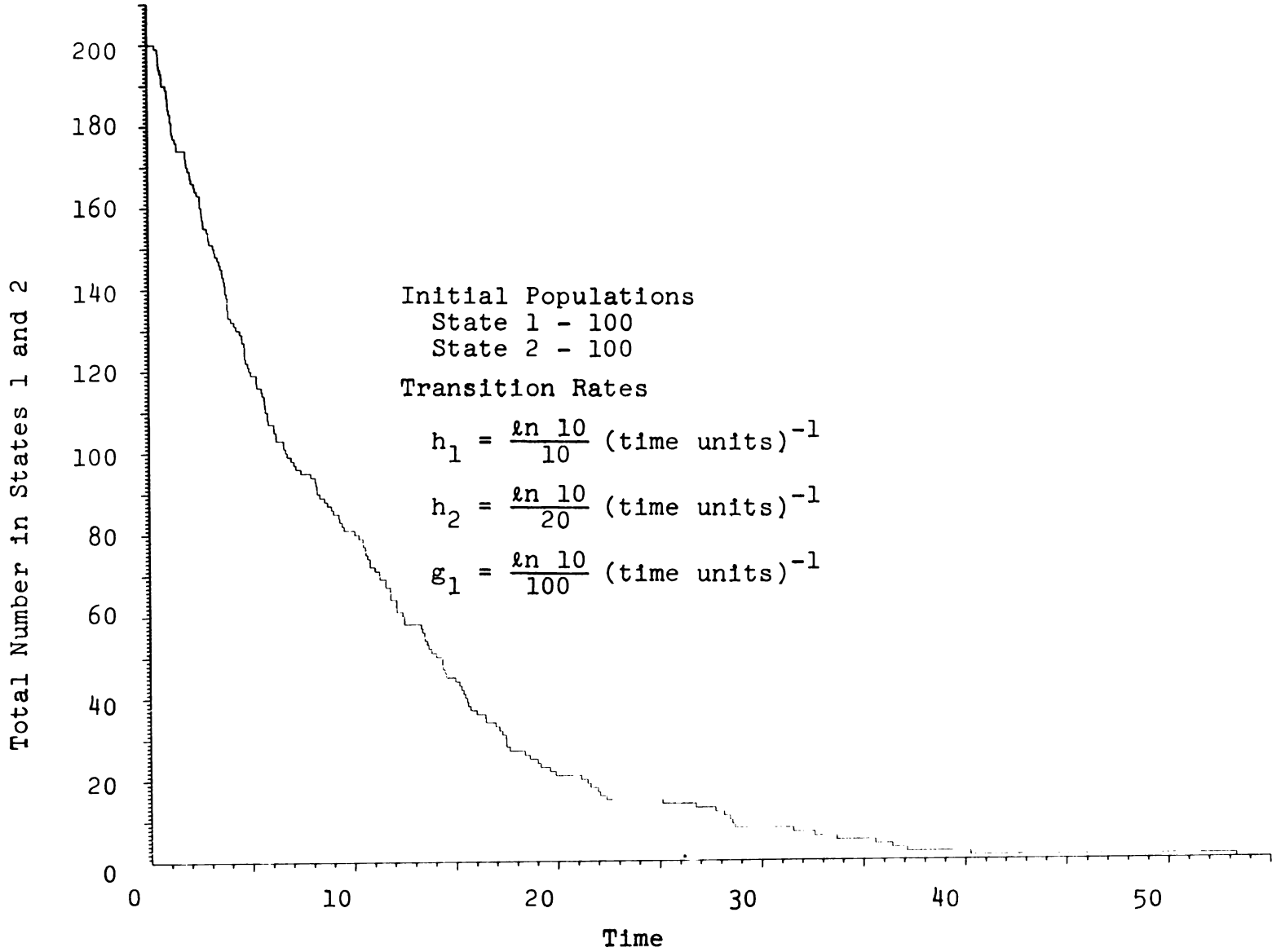

Figure 5.5.--Simulated Death Process for Model with Two Viable States--Method of Generating Time Intervals Between Each Transition.

used in Figure 5.5 and Figure 5.4 was plotted from the same program used to plot Figure 5.2.

In addition, the generating function (Equation 5.20) is the product of two binomial generating functions. So the normal approximation of the distribution may be used in the same manner as in the previous section (p. 78) to generate random data points for the distribution given there.

Analysis of Model

As compared to the deterministic model, the stochastic model provides a more accurate description of the real process. It defines the population size only at the discrete levels of possible existence and predicts the influence of random variables on the individual organisms.

The model of this chapter may be compared with the equivalent deterministic model in the same way as was done for the elementary homogeneous model of Chapter 3 (p. 41). Without giving the details of the analysis, the same conclusion as found in Chapter 3 was reached for this model. Namely, the deterministic model is sufficient for prediction of the lethal dosage required for a probability of sterility greater than 0.99.

To test the stochastic model of this chapter, the same procedure as was given for the elementary model (Chapter 3) is required. But experimental errors must be small enough so as not to overshadow the intrinsic

variation of the process. This requirement is very difficult to obtain, and it is beyond the scope of this study to search for better methods for testing micro-organism death processes.

As an example of this model, the data of Shull et al. (1962) were considered and found to have very large variations, at least too large to verify the stochastic model. However, the data were analyzed to ascertain if they fitted the generalized characteristics of the model. This resulted in the need to estimate the transition rates from the experimental data. Chapter 6 examines this problem.

CHAPTER 6

DETERMINATION OF PARAMETERS FOR TWO STATE DEATH MODEL

Statistical Methods to Determine Parameters

Before any experimental evidence of the homogeneous model of Chapter 5 can be considered, numerical values for h_1, h_2 and g_1 are required. Unless these parameters can be isolated for measurement, all three must be determined from the same set of data. In this chapter, the determination of these parameters from the same set of data is considered for the heat activation model in Chapter 5 where the experimental data would be expected to give the number in state 2. In addition to the transition parameters, the initial number in state 1, L_o, may be unknown too.

For the elementary homogeneous model (Chapter 3), only one parameter, the death rate constant, was required. This was easy to obtain by a linear regression (logarithm of population versus time). But the model considered here can not be reduced to the form of a polynomial. If

it could, standard statistical methods could be used. However, the problem of finding four parameters can be reduced to a problem of finding two parameters for certain conditions. If $h_2 < h_1$, the slope of a semilog plot of the mean of the process approaches $-h_2$ as time becomes arbitrarily large. The mean of the process was given in Equation 5.8 as:

$$\bar{m}(t) = \frac{L_o h_1}{h_1+g_1-h_2}\left[\exp(-h_2 t) - \exp(-(h_1+g_1)t)\right] + M_o \exp(-h_2 t) \quad . \tag{6.1}$$

Therefore,

$$\bar{m}(\text{large } t) = \left[\frac{L_o h_1}{h_1+g_1-h_2} + M_o\right] \exp(-h_2 t) \quad . \tag{6.2}$$

If t is zero in Equation 6.2, the ordinate intercept of the line asymptotic to the mean line for large t is obtained. Thus,

$$\text{Ordinate Intercept} = \frac{L_o h_1}{h_1+g_1-h_2} + M_o \tag{6.3}$$

Consequently, if a semilog plot of the data becomes linear for large t, h_2 can be obtained from the slope of the linear portion and one of the other three parameters determined from Equation 6.3.

Assuming that h_1 and g_1 are to be determined by statistical analysis, L_o will be determined from Equation 6.3. To find h_1 and g_1, the method of moments, the method of maximum likelihood, and the least squares method were considered. The latter method was chosen because it was the least complex of the three to apply to the model. A logarithmic transformation of the data was introduced as is usually done for models involving exponentials. Therefore, the least squares equation for this model is:

$$\text{Sum of Squares} = \sum_i \left[\ell n\ \bar{m}(t_i) - \ell n\ D(t_i) \right]^2 \qquad (6.4)$$

where $D(t_i)$ represents the experimental population size (state 2) at time t_i.

The parameters h_1 and g_1 are to be determined so that Equation 6.4 is a minimum. By taking partial derivatives of Equation 6.4 with respect to g_1 and h_1, the normal equations are obtained. Designating these equations NG and NH respectively,

$$NG(h_1,g_1) = 0 = \sum_i \left[\frac{\ell n\ \bar{m}(t_i) - \ell n\ D(t_i)}{\bar{m}(t_i)} \right] \frac{\partial \bar{m}(t_i)}{\partial g_1} \qquad (6.5)$$

and

$$NH(h_1,g_1) = 0 = \sum_i \left[\frac{\ell n\ \bar{m}(t_i) - \ell n\ D(t_i)}{\bar{m}(t_i)} \right] \frac{\partial \bar{m}(t_i)}{\partial h_1} \qquad (6.6)$$

where

$$\frac{\partial \bar{m}(t_i)}{\partial g_1} = \frac{L_o h_1}{h_1+g_1-h_2}\left[t_i \exp\left[-(h_1+g_1)t_i\right] - \frac{\left[\exp(-h_2 t_i) - \exp(-(h_1+g_1)t_i)\right]}{h_1+g_1-h_2}\right] \tag{6.7}$$

and

$$\frac{\partial \bar{m}(t_i)}{\partial h_1} = \frac{L_o}{h_1+g_1-h_2}\left[\frac{(g_1-h_2)\left[\exp(-h_2 t)-\exp(-(h_1+g_1)t_i)\right]}{h_1+g_1-h_2} + h_1 t_i \exp\left[-(h_1+g_1)t_i\right]\right] \tag{6.8}$$

Numerical Methods to Solve Normal Equations

Since Equations 6.5 and 6.6 are non-linear, explicit solutions are not possible and numerical methods are required. To simultaneously solve these non-linear equations, the two general procedures given by Hildebrand (1956, p. 450) were investigated. The first of these was the method of "successive substitutions." For this method, functions $F_1(h_1,g_1)$ and $F_2(h_1,g_1)$ were defined such that

$$h_1^{k+1} = F_1(h_1^k, g_1^k) \tag{6.9}$$

and

$$g_1^{k+1} = F_2(h_1^k, g_1^k) \tag{6.10}$$

where the superscripts indicate the number of iterations. If h_1^{k+1} and g_1^{k+1} converge to the solution of the normal equations as k increases, Equations 6.9 and 6.10 can be solved successively to reach this solution.

The functions $F_1(h_1,g_1)$ and $F_2(h_1,g_1)$ can be defined several ways to obtain a convergent series. Two common methods, the Newton-Raphson method and the method of false position (Regula Fulsi) were chosen. Ostrowski (1960) gives an extensive analysis of both of these procedures. For the method of false position:

$$F_1(h_1^k,g_1^k) = \frac{h_1^{k-1}NH(h_1^k,g_1^k) - h_1^kNH(h_1^{k-1},g_1^{k-1})}{NH(h_1^k,g_1^k) - NH(h_1^{k-1},g_1^{k-1})} \tag{6.11}$$

and

$$F_2(h_1^k,g_1^k) = \frac{g_1^{k-1}NG(h_1^k,g_1^k) - g_1^kNG(h_1^{k-1},g_1^{k-1})}{NG(h_1^k,g_1^k) - NG(h_1^{k-1},g_1^{k-1})} \tag{6.12}$$

If the Newton-Raphson method is used,

$$F_1(h_1^k,g_1^k) = h_1^k - \frac{NH(g_1^k,h_1^k)}{NH'(g_1^k,h_1^k)} \tag{6.13}$$

and

$$F_2(h_1^k,g_1^k) = g_1^k - \frac{NG(g_1^k,h_1^k)}{NG'(g_1^k,h_1^k)} \tag{6.14}$$

The second method given by Hildebrand was the generalized Newton-Raphson iteration. This technique simultaneously gives the k^{th} iteration values for h_1 and g_1. To derive this method, the nonlinear terms in a Taylor expansion of Equations 6.5 and 6.6 are neglected; and the following set of linear equations results:

$$(h_1^{k+1} - h_1^k)\frac{\partial NH(h_1^k, g_1^k)}{\partial h_1} + (g_1^{k+1} - g_1^k)\frac{\partial NH(h_1^k, g_1^k)}{\partial g_1}$$

$$= -NH(h_1^k, g_1^k) \qquad (6.15)$$

$$(h_1^{k+1} - h_1^k)\frac{\partial NG(h_1^k, g_1^k)}{\partial h_1} + (g_1^{k+1} - g_1^k)\frac{\partial NG(h_1^k, g_1^k)}{\partial g_1}$$

$$= -NG(h_1^k, g_1^k) \qquad (6.16)$$

These equations can be solved by ordinary methods for systems of linear equations.

In order that these iteration procedures converge to the required solutions for h_1 and g_1, certain conditions must be satisfied. In general, the initial approximations, h_1^1 and g_1^1, must be <u>sufficiently</u> near the true solution; and the iteration must be asymptotically stable at the true solution. Hildebrand (1956) lists several checks to determine if the latter condition will be satisfied. But no theoretical methods are known to find or describe

the area of convergence. This lack of knowledge was the major limiting factor in applying the techniques to the normal equations of the model.

Test of Numerical Procedures

To test the numerical methods developed, data were generated using the theoretical probability distribution of the stochastic model and the method outline in Chapter 5 (p. 78). Figure 6.1 illustrates the mean and some of the simulated data results for $L_o = 2*10^5$, $M_o = 10^5$, $h_1 = 0.15$, $h_2 = 0.1$ and $g_1 = 0.02$. A complete description of the generated data is given in Table 6.1. Two factors were varied to test the numerical procedures, the initial values assigned to h_1 and g_1, and the number of data points included in the analysis. Initial solutions of .005, .015, .045, and .095 were tested for g_1, and values of .1, .115, .16, and .235 were used for h_1.

The number of data points used was varied in two ways. First, time interval between data points was varied from 1 to 15 time units. Second, the span over which data points were taken was tested for two time periods: 0 to 50 and 0 to 100.

The results of these tests were twofold. Either the values of h_1 and g_1 converged to a point within several percent of the true value, or they diverged without bound. The number of data points included did not greatly affect this outcome. The use of data points in

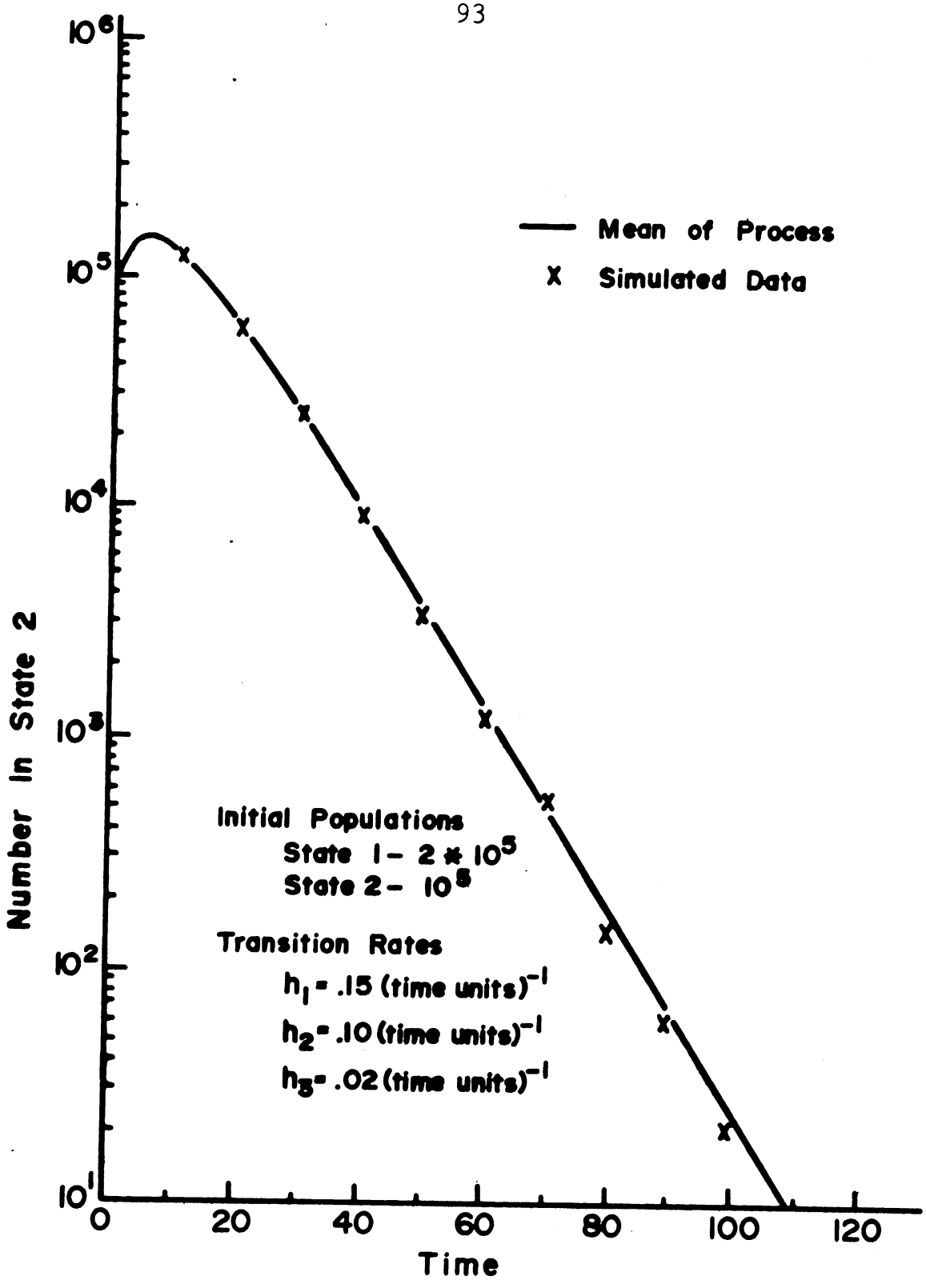

Figure 6.1.--Simulated Data for Test of Numerical Procedures.

TABLE 6.1.--Simulated data[1] to test numerical procedures.

Time	Mean of Process[2]	Generated Data Point[2]
0.0	1.000000+005	1.000000+005
1.0	1.167006+005	1.165802+005
2.0	1.277133+005	1.278511+005
3.0	1.342201+005	1.344286+005
4.0	1.371905+005	1.375992+005
5.0	1.374169+005	1.372426+005
6.0	1.355455+005	1.352477+005
7.0	1.321003+005	1.319747+005
8.0	1.275050+005	1.276065+005
9.0	1.221001+005	1.225535+005
10.0	1.161576+005	1.159621+005
11.0	1.098931+005	1.097840+005
12.0	1.034761+005	1.034640+005
13.0	9.703796+004	9.690525+004
14.0	9.067958+004	9.114750+004
15.0	8.447666+004	8.447515+004
16.0	7.848469+004	7.864076+004
17.0	7.274291+004	7.286045+004
18.0	6.727754+004	6.708961+004
19.0	6.210448+004	6.163358+004
20.0	5.723153+004	5.716327+004
21.0	5.266017+004	5.260256+004
22.0	4.838705+004	4.829220+004
23.0	4.440517+004	4.444255+004
24.0	4.070486+004	4.097648+004
25.0	3.727454+004	3.742213+004
26.0	3.410136+004	3.391315+004
27.0	3.117169+004	3.129079+004
28.0	2.847149+004	2.826854+004
29.0	2.598663+004	2.596877+004
30.0	2.370313+004	2.392843+004
31.0	2.160732+004	2.185985+004
32.0	1.968596+004	1.982628+004
33.0	1.792636+004	1.789219+004
34.0	1.631642+004	1.623829+004
35.0	1.484469+004	1.495526+004
36.0	1.350034+004	1.353512+004
37.0	1.227325+004	1.225394+004
38.0	1.115392+004	1.110343+004
39.0	1.013351+004	1.006841+004
40.0	9.203791+003	9.149679+003
41.0	8.357134+003	8.243738+003
42.0	7.586483+003	7.614369+003
43.0	6.885317+003	6.723144+003
44.0	6.247626+003	6.305551+003
45.0	5.667879+003	5.659554+003
46.0	5.140990+003	5.181749+003
47.0	4.662289+003	4.634352+003
48.0	4.227497+003	4.215839+003
49.0	3.832692+003	3.838401+003
50.0	3.474286+003	3.443167+003
51.0	3.148998+003	3.125185+003
52.0	2.853831+003	2.917185+003
53.0	2.586050+003	2.633761+003
54.0	2.343158+003	2.368917+003
55.0	2.122880+003	2.102508+003

Time	Mean of Process[2]	Generated Data Point[2]
56.0	1.923141+003	1.970404+003
57.0	1.742053+003	1.744521+003
58.0	1.577989+003	1.521233+003
59.0	1.429110+003	1.449417+003
60.0	1.294267+003	1.241873+003
61.0	1.172076+003	1.204211+003
62.0	1.061360+003	1.085119+003
63.0	9.610523+002	1.018934+003
64.0	8.701812+002	8.966196+002
65.0	7.878662+002	7.646256+002
66.0	7.133074+002	7.148970+002
67.0	6.457786+002	5.919608+002
68.0	5.846211+002	5.669627+002
69.0	5.292371+002	5.317168+002
70.0	4.790846+002	5.406635+002
71.0	4.336717+002	4.087201+002
72.0	3.925526+002	3.875082+002
73.0	3.553229+002	3.634952+002
74.0	3.216164+002	3.178051+002
75.0	2.911007+002	3.147960+002
76.0	2.634749+002	2.520887+002
77.0	2.384662+002	2.500732+002
78.0	2.158273+002	2.029398+002
79.0	1.953343+002	1.892332+002
80.0	1.767843+002	1.521595+002
81.0	1.599936+002	1.820469+002
82.0	1.447956+002	1.392428+002
83.0	1.310396+002	1.291327+002
84.0	1.185891+002	1.062414+002
85.0	1.073203+002	1.172043+002
86.0	9.712136+001	1.074102+002
87.0	8.789076+001	9.304161+001
88.0	7.953675+001	7.192436+001
89.0	7.196717+001	6.639253+001
90.0	6.513377+001	6.305014+001
91.0	5.894142+001	5.490436+001
92.0	5.333741+001	5.721963+001
93.0	4.826592+001	3.976850+001
94.0	4.367637+001	3.318353+001
95.0	3.952303+001	4.546303+001
96.0	3.576445+001	3.358390+001
97.0	3.236316+001	3.427428+001
98.0	2.928520+001	3.075848+001
99.0	2.649987+001	3.023038+001
100.0	2.397936+001	2.268864+001

[1]Initial Populations: State 1 - $2*10^5$, State 2 - 10^5.

[2]Format: First number is multiplied by 10^e, where e is the second number.

the first 50 time units gave convergence more often than if the time span of 0 to 100 time units was used. While this result might imply that additional information was a hindrance to reaching the true values of h_1 and g_1, the process for large t is a function of h_2. Therefore, the inclusion of data points between 50 and a 100 time units does not add any new information to determine h_1 and g_1. Another interesting result was that the time interval between data points had very little influence on the final outcome of the test. If the procedure converged for an interval of one time unit and a span of 50 time units, it converged for other intervals. Even for intervals of 15 time units with only three data points, the convergence was as good as for 50 data points spaced one time unit apart.

Table 6.2 gives the results for the various starting points. In cases where divergence occurred, one of two events happened. Either h_1 and g_1 increased without bound or h_1 became negative. If the latter took place, a logarithm of negative number could occur which would terminate the iteration procedure. Another solution that required termination of the iteration was if $h_1^k+g_1^k = h_2$. If this occurred, there was a division by zero in the normal equations. For certain initial values, the iteration procedures did converge to this point. When $h_1+g_1 = h_2$, a different set of equations

TABLE 6.2.--Numerical solution of normal equations for various initial conditions.

True Values: h_1=.15, g_1=.02		Numerical Solution[1]		
		Numerical Method		
Initial Values		Successive Substitutions		Generalized Newton-Raphson Method
h_1	g_1	Method of False Position	Newton-Raphson Method	
.1	.005	Convergence and Divergence	Convergence to $h_1+g_1 = h_2$	Convergence
.1	.015	Convergence	Convergence to $h_1+g_1 = h_2$	Convergence
.1	.045	Convergence	Convergence	Convergence
.1	.095	Convergence and Divergence	Divergence	Divergence
.115	.005	Convergence	Divergence	Divergence
.115	.015	Convergence	Convergence	Convergence
.115	.045	Convergence	Convergence	Convergence
.115	.095	Divergence	Divergence	Divergence
.16	.005	Convergence	Convergence	Convergence
.16	.015	Convergence	Convergence	Convergence
.16	.045	Convergence and Divergence	Convergence and Divergence	Divergence
.16	.095	Divergence	Divergence	Divergence
.235	.005	Divergence	Convergence	Divergence
.235	.015	Divergence	Convergence to $h_1+g_1 = h_2$	Divergence
.235	.045	Divergence	Divergence	Divergence
.235	.095	Divergence	Divergence	Divergence

[1]Generally the initial values led to convergence or divergence regardless of number of data points considered. Both convergence and divergence are shown where this did not hold.

apply as shown in Chapter 5. However, this was not put into the computer program because it did not seem significant in most cases.

All three numerical procedures give convergence for most initial values tested except the ones located the farthest from the true solution. But the method of false position converged over a little wider range of initial values than the other two methods. It is also the easiest to program on the computer because no derivatives are required. On the other hand, the generalized Newton-Raphson method converged the fastest. It took about one-third the computer time required for each of the two successive substitution techniques.

Application of Numerical Methods to Experimental Data

After successful use of the numerical procedures for simulated data, the techniques were applied to the experimental data reported by Shull et al. (1962). They used this data for a model[1] similar to the homogeneous two-stage model of Chapter 5. But they did not include a transition from state 1 to state 3. Without this transition, statistical techniques were not required to determine the parameters. However, the model did not give a very good fit of the data. The addition of the

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[1]See discussion on p. 69.

transition from state 1 to state 3 to the model was hoped to improve the experimental fit of the data to the theoretical model.

Table 6.3 shows the experimental data used. But convergence was not obtained for any of the three procedures successfully applied to simulated data. While in theory an area of convergence exists, in reality it could not be found even though a very large number of initial values were tested.

Two reasons can be given for this negative outcome. First, the experimental data are more irregular than the simulated data. Assuming the experimental errors were substantially reduced, the experimental data should behave similar to the simulated data and result in convergence for the parameters being estimated by the numerical procedures. But the actual process may not be the same as assumed in the theoretical model. This is a second reason for the failure to find an area of convergence for the experimental data.

Further study of this problem is needed. Perhaps the numerical procedures can be modified so that values for h_1 and g_1 can be obtained. Also, the assumption that the transitions are homogeneous can be questioned. The transition required for heat activation (state 1 to state 2) of the spore could well be time dependent. Practically no studies have been made of the

TABLE 6.3.--Experimental data for two-stage death model.[1]

Time	Population (State Two)
0	$1.26 * 10^5$
.8	$1.90546 * 10^5$
2.2	$1.41254 * 10^5$
2.6	$1.62181 * 10^5$
4.0	$1.12202 * 10^5$
4.4	$6.9183 * 10^4$
4.9	$7.0794 * 10^4$
5.4	$7.2444 * 10^4$
5.9	$4.3652 * 10^4$
6.2	$8.9125 * 10^4$
7.2	$4.4668 * 10^4$
7.8	$3.2734 * 10^4$
9.0	$7.762 * 10^3$
11.1	$7.943 * 10^3$
12.4	$1.995 * 10^3$
12.7	$5.82 * 10^2$
15.2	$1.58 * 10^2$
17.1	$1.22 * 10^2$
17.3	$7.2 * 10$
19.7	$1.4 * 10$
22.7	2
23.7	2

[1]Data taken from work of Shull et al. (1962).

mathematical nature of heat activation. From the limited data of Murrell (1961), no evidence is given that the transition is homogeneous. Further studies are needed in this regard.

CHAPTER 7

SUMMARY AND CONCLUSIONS

This study has shown how quantitative values can be assigned to the influence of random variables on the death of microorganisms. To accomplish this, axioms were stated; and a mathematical theory derived to describe real death processes. Accordingly, real processes were not studied; instead, a method of modeling was given. Modern probability theory was applied to acquire the results.

The general nature of the order of death processes of microorganisms has been considered by many people, but there were essentially no approaches from a stochastic point of view. The stochastic models given required very few assumptions in addition to the ones assumed for death rates in deterministic models.

The death processes studied were viewed as Markov processes with a continuous time parameter. The methods of deriving and solving a model of this type were illustrated for an elementary[1] process and used for a more

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[1]Organisms of a homogeneous population were assumed to either exist in a viable state or make a transition to a non-viable state.

complex model in which the organism could exist in two different viable states. In each case, a general mathematical model was derived without specifying the form of the transition parameters. From the models, a theoretical probability distribution was acquired and used to simulate the supposed process.

The elementary model was analyzed for the condition where the transition parameter was independent of time. Some experimental evidence that the model predicted the experimental derivation was found, but no conclusive verdict can be made without further study and better instrumentation to minimize experimental errors.

For the practical problem of sterilizing populations of microorganisms, deterministic models were found to give a good approximation of the stochastic model.

The estimation of parameters for the model with two viable states was considered. A complex system of non-linear equations ensued. Numerical methods were developed to simultaneously solve these equations. But their application was only successful for simulated data. Further work is needed to refine these procedures for use with experimental data.

The methods and results of this study could be extended to a model with n different viable states during a death process. In general, the use of stochastic models should be considered in other engineering modeling studies where biological entities are involved. By

using a stochastic approach, experimental variation from the mean is not viewed as experimental error. Instead, variations can be predicted, and the stochastic model can quantitatively describe the expected size of these deviations from the mean.

APPENDICES

APPENDIX A

STATIONARY TRANSITION PROBABILITIES

From Equation 1.7 of Chapter 1,

$$p_{ki}(u,s) = \sum_{j=1}^{N} p_{kj}(u,t)p_{ji}(t,s). \tag{A.1}$$

When the transition probabilities depend only on the length of the time interval and not on its starting point,

$$p_{ji}(t,s) = p_{ji}(t-s) \tag{A.2}$$

and

$$p_{ki}(u,s) = p_{ki}(u-t+t-s) \tag{A.3}$$

This can be written as:

$$p_{ki}(u,s) = \sum_{j=1}^{N} p_{kj}(u-t)p_{ji}(t-s) \tag{A.4}$$

By redefining the time intervals as $t = u-t$, $s = t-s$ and substituting into Equation A.4,

$$p_{ki}(s+t) = \sum_{j=1}^{N} p_{kj}(t)p_{ji}(s) \tag{A.5}$$

APPENDIX B

LAGRANGE'S METHOD FOR SOLUTION OF A PARTIAL DIFFERENTIAL EQUATION OF FIRST ORDER

The stochastic models in this thesis yield first order partial differential equations. This is a common type of equation for many Markov processes. The best method of solution of this type of equation was given by Lagrange. For an account of the general theory, the texts by Cohen (1933) and Forsyth (1885) can be consulted.

In order to find a solution to a partial differential equation of the form:

$$R_1 \frac{\partial z}{\partial x_1} + R_2 \frac{\partial z}{\partial x_2} + R_3 \frac{\partial z}{\partial x_3} = Q \qquad \text{(B.1)}$$

where R_1, R_2, R_3 and Q are functions of the independent variables x_1, x_2, x_3 and the dependent variable z, consider the linear partial differential equation

$$R_1 \frac{\partial \theta}{\partial x_1} + R_2 \frac{\partial \theta}{\partial x_2} + R_3 \frac{\partial \theta}{\partial x_3} + Q \frac{\partial \theta}{\partial z} = 0 \qquad \text{(B.2)}$$

If this equation has solution $\theta(x_1,x_2,x_3,z) = C$ where C is a constant of integration, then this equation can be solved in terms of z giving a solution of Equation B.1. Therefore, a solution for Equation B.2 leads to a solution of Equation B.1.

To solve Equation B.2, Lagrange showed that a system of ordinary differential equations could be formed

which would have the same solution as Equation B.2. These so-called subsidiary equations are formed in the following way:

$$\frac{dx_1}{R_1} = \frac{dx_2}{R_2} = \frac{dx_3}{R_3} = \frac{dz}{Q} \tag{B.3}$$

Now the problem has been reduced to solving a series of ordinary differential equations. From Equation B.3, choose three independent equations which can be integrated. Let the three solutions to these integrals be

$$\theta_1(x_1,x_2,x_3,z) = b_1 \tag{B.4}$$

$$\theta_2(x_1,x_2,x_3,z) = b_2 \tag{B.5}$$

$$\theta_3(x_1,x_2,x_3,z) = b_3 \tag{B.6}$$

where b_1, b_2 and b_3 are constants of integration.

Then any arbitrary function $W(\theta_1,\theta_2,\theta_3) = 0$ with partial derivatives will be the general solution of Equation B.2. In this case, choose an arbitrary function such that:

$$Z(\theta_1,\theta_2) = \theta_3 \tag{B.7}$$

which is a more convenient form of the solution. The function Z can be explicitly determined if initial conditions are given.

Assuming initial condition:

$$Z = \zeta(x_1,x_2,0), \tag{B.8}$$

then substitute Equation B.8 into Equation B.7.
Therefore,

$$Z(\theta_1(x_1,x_2,0,\zeta(x_1,x_2,0)),\theta_2(x_1,x_2,0,\zeta(x_1,x_2,0))$$

$$= \theta_3(x_1,x_2,0,\zeta(x_1,x_2,0)) \tag{B.9}$$

To determine the exact form of Z, let:

$$\lambda_1 = \theta_1(x_1,x_2,0,\zeta(x_1,x_2,0)) \tag{B.10}$$

and

$$\lambda_2 = \theta_2(x_1,x_2,0,\zeta(x_1,x_2,0)) \tag{B.11}$$

From these two equations, solve for x_1 and x_2 in terms of λ_1 and λ_2. This result is then substituted into Equation B.9, and the function Z is defined. The general solution is then obtained from Equation B.7 with $\lambda_1 = \theta_1$ and $\lambda_2 = \theta_2$ where θ_1 and θ_2 are defined in Equations B.4 and B.5. Thus $\theta_3 = Z(\theta_1,\theta_2)$ has been completely specified, and the original partial differential equation solved yielding a useful relationship between x_1, x_2, x_3 and z.

In the foregoing the method of Lagrange was illustrated for the case of three independent variables. An extension to any number of independent variables follows the illustrated procedure along exactly the same lines.

APPENDIX C

SOLUTION OF DIFFERENTIAL EQUATION FOR STOCHASTIC MODEL WITH INTERMEDIATE STATE

The stochastic model of Chapter 4 yielded the following exact differential equation:

$$\frac{d\Psi(x,y,t)}{0} = \frac{dx}{yh_1(t) + g_1(t) - (h_1(t) + g_1(t))x}$$

$$= \frac{dy}{h_2(t)(1-y)} = \frac{dt}{-1} \qquad (C.1)$$

It immediately follows that

$$\Psi(x,y,t) = a_1. \qquad (C.2)$$

In the above equation and in the following ones, the a_1's stand for some constant. From Equation C.1, the last two differentials can be solved as follows:

$$\frac{dy}{h_2(t)(1-y)} = \frac{dt}{-1} \qquad (C.3)$$

This has solution:

$$\ell n(y-1) = \int_o^t h_2(\tau)d\tau + a_2 \qquad (C.4)$$

And it can be written:

$$y = 1 + a_3 \exp\left(\int_o^t h_2(\tau)d\tau\right) \qquad (C.5)$$

or

$$a_3 = (y-1)\exp\left(-\int_o^t h_2(\tau)d\tau\right) \qquad (C.6)$$

The stochastic model of Chapter 4 yielded the following exact differential equation:

$$\frac{d\Psi(x,y,t)}{0} = \frac{dx}{yh_1(t) + g_1(t) - (h_1(t) + g_1(t))x}$$

$$= \frac{dy}{h_2(t)(1-y)} = \frac{dt}{-1} \qquad (C.1)$$

It immediately follows that

$$\Psi(x,y,t) = a_1. \qquad (C.2)$$

In the above equation and in the following ones, the a_i's stand for some constant. From Equation C.1, the last two differentials can be solved as follows:

$$\frac{dy}{h_2(t)(1-y)} = \frac{dt}{-1} \qquad (C.3)$$

This has solution:

$$\ell n(y-1) = \int_o^t h_2(\tau)d\tau + a_2 \qquad (C.4)$$

And it can be written:

$$y = 1 + a_3 \exp(\int_o^t h_2(\tau)d\tau) \qquad (C.5)$$

or

$$a_3 = (y-1)\exp(-\int_o^t h_2(\tau)d\tau) \qquad (C.6)$$

Now a second pair of independent differentials from Equation C.1 can be chosen and solved. For

$$\frac{dx}{yh_1(t) + g_1(t) - (h_1(t) + g_1(t))x} = \frac{dt}{-1} \quad , \qquad (C.7)$$

substitute the expression found for y in Equation C.5 and rearrange the expression. Then,

$$\frac{dx}{dt} - \left(h_1(t)+g_1(t)\right)x = -g_1(t) - \left(1 + a_3\exp(\int_o^t h_2(\tau)d\tau\right)h_1(t)$$

(C.8)

This is a linear differential equation with solution:

$$(x-1)\exp\left(-\int_o^t (h_1(\tau) + g_1(\tau))d\tau\right) + (y-1)\exp\left(-\int_o^t h_2(\tau)d\tau\right) *$$

$$\int_o^t h_1(\tau)\exp\left(-\int_o^t [h_1(\tau) + g_1(\tau) - h_2(\tau)]d\tau\right) = a_4$$

(C.9)

Using Equations C.2, C.6 and C.9, $\Psi(x,y,t)$ can be defined as a function of a_3 and a_4.

$$\Psi(x,y,t) = f(a_3,a_4) \qquad (C.10)$$

Given the initial conditions,

$$\Psi(x,y,0) = x^{L_o} y^{M_o} \quad . \qquad (C.11)$$

Using the form given in Equation C.10 for t = 0,

$$f(y-1,x-1) = x^{L_o} y^{M_o} \tag{C.12}$$

In order to define this function for solution of general form in Equation C.10, let

$$\rho_1 = y - 1 \tag{C.13}$$

$$\rho_2 = x - 1 \tag{C.14}$$

Then Equation C.12 can be written:

$$f(\rho_1,\rho_2) = (\rho_2+1)^{L_o}(\rho_1+1)^{M_o} \tag{C.15}$$

Then from Equation C.10,

$$\Psi(x,y,t) = (a_3+1)^{M_o}(a_4+1)^{L_o} \tag{C.16}$$

This can be rewritten as:

$$\Psi(x,y,t) = [y\alpha+1-\alpha]^{M_o}[x\beta+y\gamma+1-\beta-\gamma]^{L_o} \tag{C.17}$$

where

$$\alpha = \exp\left(-\int_o^t h_2(\tau)d\tau\right) \tag{C.18}$$

$$\beta = \exp\left(-\int_o^t [h_1(\tau)+g_1(\tau)]d\tau\right) \tag{C.19}$$

$$\gamma = \alpha\int_o^t h_1(t)\exp\left(-\int_o^t [h_1(\tau)+g_1(\tau) - h_2(\tau)]d\tau\right) \tag{C.20}$$

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