A MATHEMATICAL MODEL OF NORMAL GRANULOCYTE KINETICS

by

Blanche Louise McFarland

Institute of Statistics
Mimeograph Series No. 1197
Raleigh, NC September 1978

ABSTRACT

McFARLAND, BLANCHE LOUISE. A Mathematical Model of Normal Granulocyte Kinetics. (Under the direction of H. R. VAN DER VAART).

The purpose of this study was to determine if there is a feedback mechanism operating on the production and development of granulocytes; and if so, how the feedback mechanism operates. The maturation and development of granulocytes have been partitioned into three compartments: the mitotic compartment, the storage compartment, and the blood. Twenty per cent and 40 per cent neutropenia in the blood were achieved by causing the blood to lose approximately 20 per cent or 40 per cent of its cells. Mathematical models were developed to simulate on the computer two different feedback mechanisms. One model is designed so that release of cells from storage to the blood is dependent upon the number of cells in the blood, and the release of cells from the mitotic compartment to the storage compartment is dependent upon the number of cells in storage. The other feedback mechanism is modelled so that release of cells from the mitotic compartment to the storage compartment, and release of cells from storage to the blood depend upon the number of cells in the blood.

Results indicate that a feedback mechanism is operating on the granulocyte system. When neutropenia is simulated either feedback mechanism causes an increase of cells in the blood to near or above the steady state value within 0.2 of an hour after neutropenia is achieved. Around 5 hours after onset of neutropenia the number of blood granulocytes overshoots the steady state value and returns to normal within less than an hour after this overshoot. These simulated

results are closely similar to experimental behavior of blood granulocytes after neutropenia has been achieved.

Since both models give basically the same results in the blood granulocyte pool, it is difficult, at this particular point of the study, to ascertain which feedback model better parallels the biological system.

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BLANCHE LOUISE McFARLAND

A thesis submitted to the Graduate Faculty of
North Carolina State University at Raleigh
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

DEPARTMENT OF STATISTICS

RALEIGH

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APPROVED BY:

Harvey J. Gold — Richard D Walker

James Penny — H. R. van der Vaart

Chairman of Advisory Committee

BIOGRAPHY

Blanche Louise McFarland was born June 13, 1946, in Houston, Texas. She completed her primary and secondary education in Houston, receiving her high school diploma in 1964 from Jack Yates Senior High School. From 1964 to 1968 the author studied Biology at Texas Southern University in Houston where she earned the Bachelor of Science Degree in Biology.

From September 1969 to June 1971 the author studied Microbiology at Atlanta University in Atlanta, Georgia. It was during her interim at Atlanta University that she became interested in Biomathematics. She worked as a research assistant to Dr. Judith Rae Lumb, Associate Professor of Microbiology, during the 1970-71 school year. The author's research involved developing a computer model for lymphocyte production in the thymus. In 1971 she received the Master of Science Degree in Biology from Atlanta University.

The author has been a trainee in the Biomathematics Program of the Department of Statistics at North Carolina State University since July 1971. In 1974 and 1976 she served as Visiting Lecturer of Mathematics at North Carolina Central University.

ACKNOWLEDGEMENTS

Completing a program of study is very arduous and in my case could not have been accomplished without the encouragement, help, and advice of many people.

During the entirety of my study in the Biomathematics program I was supported for the most part by a Biomathematics training grant from the National Institute of Health. I would therefore like to express my gratitude for this source of financial aid and education. Professor H. L. Lucas, Director of the Biomathematics Program prior to his death in 1977, served as a member of my advisory committee. Even though the period of time that he served on my committee was brief, I am very thankful for his advice and encouragement. Professor H. R. van der Vaart, in addition to serving as Chairman of my committee and advisor to my research project, aided me in developing the mathematical and statistical background that I lacked due to my training as a biologist. He has been more than patient and understanding and through his teaching, as well as through mathematics courses I have taken at this university, I feel that I have learned the necessary mathematics that has made me functional in biomathematics. In this regard I would like to express my gratitude and give a very special word of thanks to Professor van der Vaart.

Some of the experimental data for my research were obtained from Dr. R. I. Walker. In addition, as a member of my committee Dr. Walker has been a valuable source for information regarding the medical and biological aspects of my dissertation project.

I would like to thank Professor Jerome Perry and Professor Harvey Gold, the other two members of my committee, for their help, encouragement, and ideas pertaining to the research and dissertation.

My family has been very instrumental in aiding me to complete my coursework and research. My mother, Isabella T. McFarland, has been a source of inspiration and comfort during the entirety of my studies. My father, Cyrus O. McFarland, has been a reliable and continuous source of financial supplement to the support that I received from the National Institute of Health. Both my sister, Willie Mae Lewis, and my brother, Cyrus O. McFarland, Jr., have been a reservoir of advice and friendship in times of need. Therefore, I would like to extend my appreciation to my family and to express my deepest thanks to each and every one of them.

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1. INTRODUCTION

1.1 Definition of the Biomathematical Problem

The study of white blood cells is not new as evidenced by such works as those completed by Osgood (1950) and Ottesen (1954). In particular, granulocytes have received an enormous amount of attention and study. Patt and Maloney (1959) and Cronkite and Vincent (1970) have been among numerous scientists to investigate the production and development of granulocytes. Despite the attention devoted to the kinetics of granulocytes, there still remains some controversy concerning these cells. Investigators are in disagreement concerning the mechanics involved in granulocyte production and development; presently there are several proposed mechanisms for the control of granulocyte production, development, and release into the circulating blood.

The objective of this dissertation project is to study mathematically and probabilistically the kinetics of the maturation stages of the particular type of white blood cells called granulocytes. The maturation process includes the development of the cells in the bone marrow through their introduction into the circulating blood. Mathematical equations have been formulated that result in computer simulation data that parallel the experimental data obtained by Patt et al. (1957, p. 586) of the recovery of the dog's blood neutrophils after induction of neutropenia. The model developed in this study will serve as an intermediate model that will later be modified to produce computer simulation data that parallel the orthoradiophosphate

experimental data on the horse obtained by R. I. Walker. The model presented here is intended to be an extension of the granulocyte kinetic project completed by O'Fallon, Walker, and van der Vaart (1971).

1.2 Historical Background

O'Fallon's mathematical work was prompted by experimental work done by Walker on the granulocytes of the rabbit, horse, and man. The history of this experimental work can be traced to Walker et al. (1960, 1961, 1962, 1964, 1965). O'Fallon cited Otteson (1954), Kline (1959), Patt and Maloney (1959), and Cronkite et al. (1960) as having done experimental work of the same type as Walker on the same leucocyte system. Rigas (1962), Warner and Athens (1964), and Patt and Maloney (1964) were noted by O'Fallon as having developed models of some sort that describe granulocyte kinetics.

Review of related literature reveals that several investigators (Cronkite and Vincent, 1970; Vogler et al., 1972; Morley et al., 1970; and Lajtha et al., 1964) are of the opinion that there is a feedback mechanism of some sort operating on the production and development of granulocytes. They propose that when the number of cells in the blood falls below a certain threshold level a signal, possibly chemical, is relayed to the storage (the compartment where cells migrate after cell division or mitosis) and mitotic compartments to synthesize and release needed cells to the blood. Experimental studies of the horse's granulocytes obtained by Walker indicate that a feedback may be operating on the cells. His experimental

studies reveal instances when the horse had long lag periods where no labelled cells entered the blood, followed by a sharp increase in the number of labelled granulocytes entering the circulating blood.

1.3 Proposed Method of Modelling the Biological System

Since the literature is obscure as to how the feedback mechanism operates, two different feedback models have been developed and studied in this thesis. The first model is one where the storage compartment is dependent upon the cells in the blood and will release cells to the circulating blood when the blood granulocyte pool needs to be replenished; and the mitotic compartment is dependent upon the storage to release cells to the storage compartment. The second feedback was modelled so that both the storage and mitotic compartments are directly dependent upon the number of cells in the circulating blood. When the circulating blood loses granulocytes, a signal is imparted to both the storage compartment to release needed cells to the blood, and to the mitotic compartment to synthesize new cells, simultaneously. The two mechanisms will be discussed and compared to determine which mechanism best fits the data of Patt et al. (1957). See Figures 1.3.1 and 1.3.2 following this discussion for illustrations of the two mechanisms.

Before incorporating a feedback mechanism into O'Fallon's model, his equations have first been modified to simulate a strict steady state condition in accordance with the experimental data obtained by Cronkite and Vincent (1970). The steady state condition is then altered to produce approximately 20 per cent neutropenia by

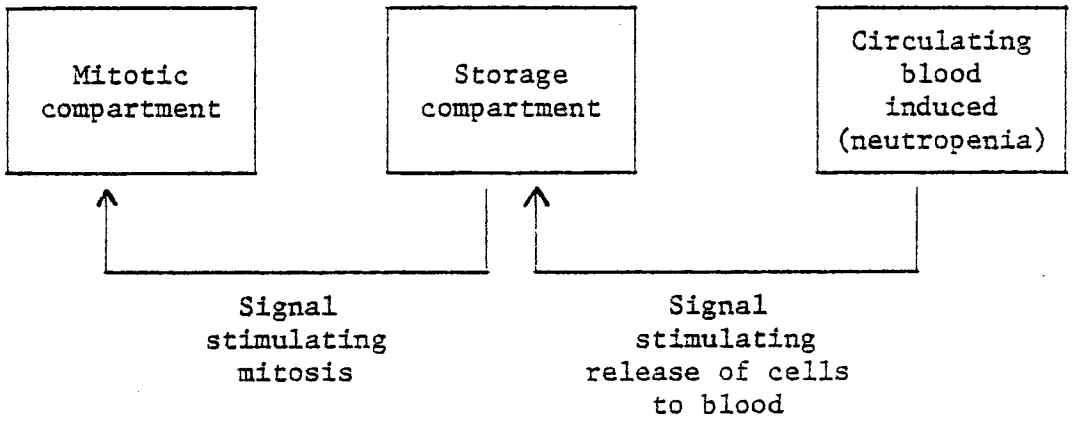

Figure 1.3.1 Cascading of feedback to storage and mitotic compartment

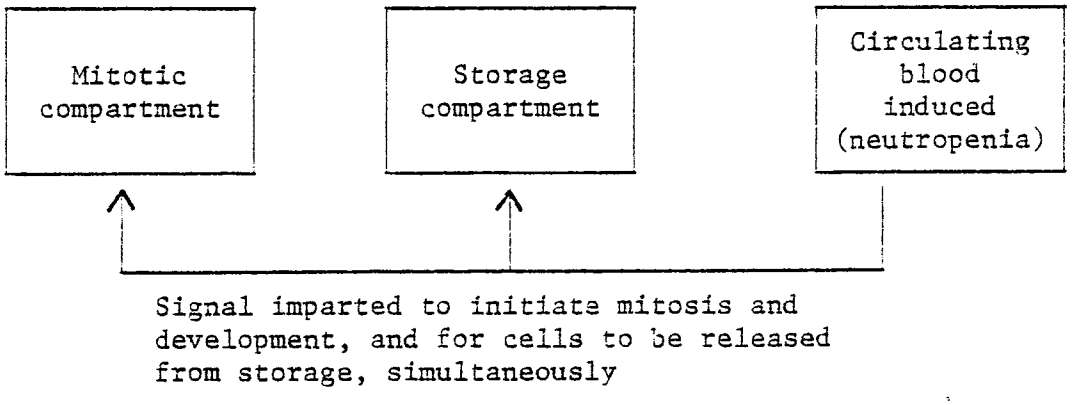

Figure 1.3.2 Simultaneous feedback to storage and mitotic compartment

lowering the normal level of blood granulocytes by the appropriate percentage. The data used for simulation of neutropenia were obtained from Patt and Maloney (1957). O'Fallon's mathematical equations are then changed to cause the storage compartment to respond to the loss of cells in the blood, as well as to cause the mitotic compartment to respond to loss of cells in either the blood or the storage compartment. It should be mentioned that the data obtained from Cronkite and Vincent (1970) are experimental results obtained from man, whereas those obtained by Patt et al. (1957) are experimental results from the dog. The parameter values extracted from these two studies are only used as initial values to get the computer programs workable. The final parameter values will be chosen as those that best fit the experimental data obtained by Walker (1973) on the horse.

1.4 Outline of the Specific Experiment and Results of Patt and Maloney

Since my thesis essentially involves developing a model that fits the data of Patt et al., this introductory chapter will conclude with a brief outline of the experiment and results of Patt et al. (1957).

The experiment was performed with mongrel dogs of either sex. The dogs were isolated and immunized against various diseases. After the isolation period total and differential leucocyte counts were made on the dogs' blood (blood samples were obtained from the external jugular vein by venipuncture). Total leucocyte counts were made in duplicate using two pipettes. Differential leucocyte counts were

made on cover glass smears prepared with Wright's stain, a minimum of 200 cells being enumerated on each smear wherever possible.

Various doses of a leucocyte antiserum were injected intravenously to achieve different degrees of neutropenia. The antiserum was not specific for neutrophils, but had lesser effect on leukocytes other than neutrophils. Periodic samples of the blood were then taken and the amount of leucocytes was determined by the previously mentioned counting methods.

The experimental results are expressed as relative change in blood neutrophil count per time (in hours).

Results of this study revealed that the severity of neutropenia increases with increasing dose of antiserum. The maximum amount of neutropenia varies inversely with the concentration of antiserum. Figure 1.4.1 on the next page shows the relative change in blood neutrophil count after intravenous injection of variable concentrations of leucocyte antiserum into 12 dogs.

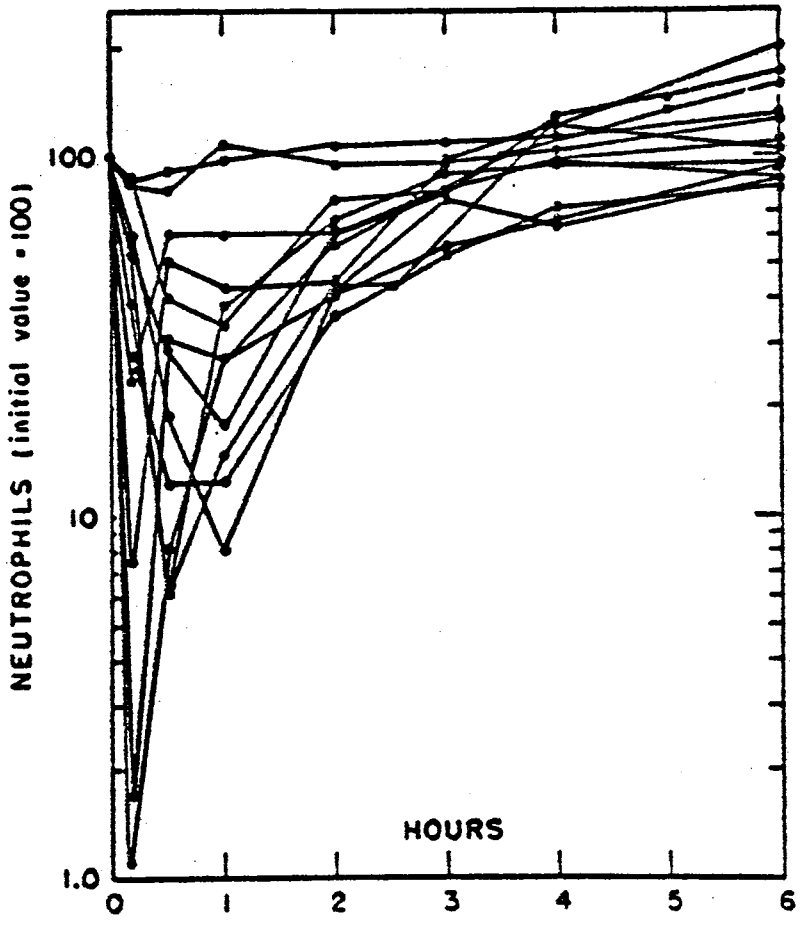

Figure 1.4.1 The relative change in blood neutrophil count after intravenous injection of variable concentrations of leucocyte antiserum (Reprinted from Patt et al., 1957, p. 586)

2. REVIEW OF THE BIOLOGICAL SYSTEM AND SUMMARY OF O'FALLON'S MODEL

2.1 The Biological System

As mentioned in the introduction, the model developed is one for the production and development of a particular type of white blood cell, viz., granulocytes. White blood cells in general are known as leucocytes and are distinguished from the red blood cells, erythrocytes, in many respects. Some major differences are that red blood cells contain hemoglobin, are non-nucleated, and spend their life span in the bloodstream. The white blood cells contain no hemoglobin, are nucleated, and are capable of ameboid movement which enables them to pass through blood vessels. Leucocytes play a major role in aiding an organism against bacteria.

The types of leucocytes are the lymphocytes, monocytes, neutrophils, eosinophils, and basophils. Neutrophils, eosinophils, and basophils are the granulocytes, and are characterized by numerous granules. Lymphocytes and monocytes contain granules, but these granules differ from those of the granulocytes. According to Bailey (1964, pp. 131-146) neutrophils are polymorphonuclear leucocytes varying in size from 9-12 μ. They constitute about 60-70 per cent of the total white blood cells. Eosinophils are characterized by a large number of coarse refractile granules that are highly stainable with eosin or acid dyes. Eosinophils represent about 2-4 per cent of the leucocytes. The basophils constitute from 0.5-1.0 per cent of the leucocytes. They range in size from 9-10 μ. They possess a large irregular polymorphous nucleus.

It should be mentioned that the lymphocytes and monocytes constitute about 20-25 per cent and 3-8 per cent of the leucocytes, respectively. Inherent in the preceding percentages is the fact that neutrophils must represent over 95 per cent of the granulocytes. Because the neutrophils represent the bulk of the granulocytes, the feedback will basically be a study of neutrophils.

The mature granulocyte, once it reaches the circulating blood, has gone through a series of maturation and differentiation stages. The stages of granulocyte development in order of increasing maturity are stem cell, myeloblast, promyelocyte, myelocyte, metamyelocyte, band granulocyte, and segmented granulocyte. The stem cells, myeloblasts, promyelocytes, and some myelocytes (the dividing myelocytes) are called the dividing cells. These cells are located in what O'Fallon calls the mitotic compartment. This is essentially the portion of the bone marrow where mitosis takes place. It is assumed that only cells preparing for mitosis or cells that are actually undergoing mitosis are in this compartment. After a cell completes its final division it enters what is known as the post-mitotic or storage compartment; this is the portion of the bone marrow where cells undergo maturation by differentiation (no mitosis or cell division takes place here). The cells in this non-dividing storage compartment are the non-dividing myelocytes, metamyelocytes, the band and segmented granulocytes. The storage and mitotic compartments are physiologic compartments occupying the same geographic location.

According to Cronkite and Vincent (1970, pp. 218, 219) the minimum transit times through the storage compartment are 3 hrs. for

myelocytes to metamyelocytes, 36 hrs. to the band neutrophils, and 48 hrs. to the segmented neutrophils. They also indicate that there is an obligatory minimum time of 48 hrs. for a cell to mature from the last myelocyte division to a marrow granulocyte; also, that under normal conditions the mature granulocytes (segmented) are the only ones available to be drawn on in need, but that under stress of great need, non-segmented granulocytes (band forms and metamyelocytes) immediately enter the blood.

Thus far I have mentioned such biological terms as mitosis and DNA synthesis. I think it is useful for the non-biologist that I give a brief discussion of the biology of mitosis.

Every dividing cell has what is known as a generation time. Generation time is simply the time it takes for a cell's formation until it has completely divided forming two daughter cells. Generation time is divided into four subphases (see Lobue, 1970).

1. Mitosis--the actual process of cell division. DNA synthesis must occur earlier. Mitosis is the time of chromosomal formation and the actual division of the cell. During prophase the DNA--new and old--forms into chromosomes; these line up across the middle of the nucleus in pairs--one old, one new--and then split apart.
2. G1 phase--the postmitotic presynthetic rest period or phase. This is the phase where the cell is recovering from the previous mitosis. The cell is undergoing physical reorganization and return to non-mitotic form. The thesis acts on the assumption that the feedback acts on this phase.

It will be explained in Chapter 4 how the feedback is thought to operate on this phase.

3. S phase--this is the phase where DNA is synthesized. During this phase the cell incorporates DNA precursors. Watson (1970, pp. 255-353) proposes that during this time the hydrogen bond of the DNA molecule is broken and the double strand is separated. Each strand then serves as a template for the synthesis of a new complementary strand. That is, each base in a strand would attract the particular base that it is known to pair with (A-T.C-G). The final result would be two double stranded DNA molecules.
4. G2 phase--the postsynthetic premitotic rest period. During this phase chromosomes are in preparation for mitosis.

2.2 O'Fallon's Model

O'Fallon, in his model, partitioned the maturation and development of granulocytes into three compartments: the mitotic compartment (bone marrow) where cells divide synthesizing new DNA and incorporating label; the storage compartment (bone marrow) where labelled cells migrate after completing mitosis to undergo further differentiation; and the circulating blood where the granulocytes migrate after a sojourn in the storage compartment. Using this partitioning, O'Fallon then formulated a mathematical model that yielded computer data that coincided with the experimental data obtained by Walker's orthoradiophosphate study of the rabbit's granulocyte production and development. There are, however, some problems regarding the experimental data obtained from the horse.

O'Fallon defined the amount of label incorporated in the DNA of the circulating granulocytes at any time t as the specific activity of the DNA-TE of circulating granulocytes at any time t. His model for this specific activity can best be summarized by the following theorem: Assume a granulocyte arrives in the circulation at time $w \leq t$ after having spent x units of time in storage. Assume at the time of replication each granulocyte contains one unit of DNA-Tracer (DNA-TE). Then b(t), the expected specific activity of DNA-TE of circulating granulocytes at time t, is

$$\begin{cases} 0 \quad , \quad \text{if } t \leq 0 \\ \dfrac{\int_0^t f(x) \int_0^{t-x} a(z)[1-K(t-x-z)]\,dz\,dx}{\int_{-\infty}^{t} [1-K(t-w)\,dw]} \quad , \quad \text{if } t \geq 0 \end{cases}$$

(a) x = the sojourn time of a granulocyte in the storage compartment. O'Fallon and Walker assumed that the sojourn time of a granulocyte in the storage compartment is a random variable. Walker's experimental result indicates that some cells must exit storage soon after entering. On the basis of this O'Fallon proposed two populations (or rather two subpopulations) of cells in storage in proportions π and $1 - \pi$). Therefore, the probability density function f(x) of the sojourn time x is defined by the following expression:

$$f(x) = \pi f_1(x) + (1 - \pi) f_2(x)$$

where $f_1(x) = \gamma \exp[-\gamma x]$, $\gamma > 0$ and $f_2(x) = \dfrac{1}{\sigma\sqrt{2\pi}} \exp -\tfrac{1}{2} \{\dfrac{x-\mu}{\sigma}\}^2$, $\mu, \sigma > 0$.

Both f_1 and f_2 are probability functions. For further discussion see O'Fallon (1967, pp. 32, 55, 68-70).

(b) A(z) = expected specific activity of the DNA - TE of cells entering storage. Since the model developed in this thesis for the feedback does not include tracer, a detailed description of A(z) is not necessary.

(c) K(t) = the sojourn time of a granulocyte in the circulating blood. O'Fallon assumes this sojourn time to be a random variable. He also assumes that the cells leave the circulating blood according to a Poisson process. He represents the probability distribution function of the sojourn time of granulocytes in the circulation by:

$$K(t) = 1 - \exp[-\xi t], \quad \xi > 0 .$$

The proof of this theorem will not be covered in this thesis. For derivation of b(t) see O'Fallon (1967, pp. 46-49). The graph illustrated in Figure 2.2.1 is the one that is most representative of the experimental data. For this curve the following parameter values were used:

1. $\xi = .5$.
2. $\gamma = .05$.
3. $\mu, \sigma = (57, 2)$.
4. $\pi = .18$.

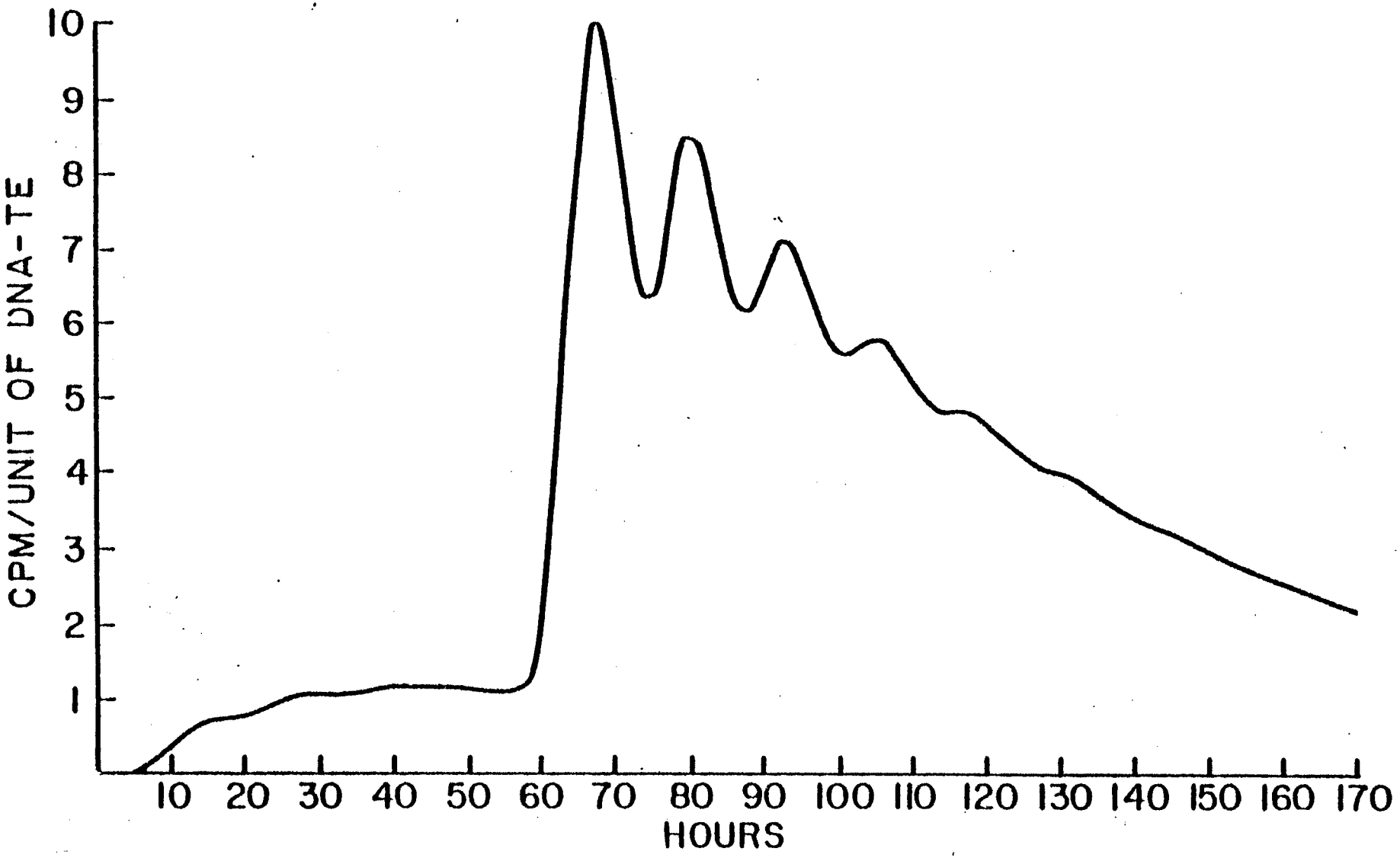

Figure 2.2.1 Expected specific activity of DNA-TE of circulating granulocytes at time t (Reprinted from O'Fallon, 1967, p. 164)

3. DERIVATION OF THE MODEL

3.1 Derivation of the Continuous Model

It was explained in Section 2.2 that O'Fallon defined the distribution of sojourn time in storage by two processes--one for cells whose sojourn is distributed normally and one for cells whose sojourn is distributed exponentially. Denoting by f either the normal or the exponential density function,

$$(3.1.1) \qquad F(T) = \int_0^T f(t)dt$$

is the cumulative distribution of sojourn time in storage for both the normal and the exponential cases.

The conditional probability that a cell will leave storage after spending between T and T + k units of time in storage given that the cell has spent T units of time in storage is:

$$(3.1.2) \qquad \frac{\int_T^{T+k} f(t)dt}{1-F(T)} \sim \frac{f(T)k}{1-F(T)} \quad .$$

This represents the fraction of cells of age[1] T that will leave storage before they reach age T + k.

Let M(t,T)dT represent the number of cells whose sojourn time in storage is between T and (T + dT) at time t. Then,

$$(3.1.3) \qquad M(t,T)dT\,\frac{f(T)k}{1-F(T)}$$

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[1] "Age" will be used synonymously with "sojourn time."

cells will leave storage and enter the blood before they are between (T + k) and (T + k + dT) units old. This results in the equation

$$(3.1.4) \qquad M(t+k,\ T+k)dT - M(t,T)dT = -M(t,T)dT\ \frac{f(T)k}{1-F(T)}$$

$$\text{so,} \qquad \lim_{k\to 0} \frac{1}{k}\ [M(t+k,\ T+k) - M(t,T)] = -M(t,T)\ \frac{f(T)}{1-F(T)}\ .$$

This is a partial differential equation for M whose solution is:

$$M(t,T) = [1-F(T)]M(t-T,0)$$

(see Rubinow and Lebowitz, 1974). Here M(t-T,0) is the rate of entry of cells into storage, comparable to the total birth rate in demography.

It was mentioned in Section 2.2 that Cronkite and Vincent (1970) suggest that cells must spend a minimum of 48 hours in the storage compartment. O'Fallon (1967) believes that there is a subpopulation of cells that have a minimum obligatory time in storage that seems to be around three hours for the rabbit. Patt et al. (1957) produced experimental evidence showing that an increase of non-segmented neutrophils occurred in the blood after neutropenia was achieved in the blood of the dog. They found that the ratio of non-segmented to segmented neutrophils reached its highest peak at about three hours after neutropenia. Therefore, it will be assumed M(t,T) has two forms.

For the normal density function

$$M(t,T) = [1-F(T)]M(t-T,0)$$

will be given by

$$(3.1.5)\quad \begin{cases} M(t,T) = \\ \end{cases} \begin{cases} M(t-T,0)\ , & \text{if } T \leq 48 \\ M(t-T,0)\left(1 - \int_0^T f(t)dT\right)\ , & \text{if } T > 48 \end{cases}$$

where $$f(t) = \frac{1}{\sigma\sqrt{2\pi}}\, e^{-\frac{1}{2}\left(\frac{t-\mu}{\sigma}\right)^2}\ , \quad \mu = 4.5\sigma = 48\ .$$

Similarly, for the exponential density function:

$$(3.1.6)\quad \begin{cases} M(t,T) = \\ \end{cases} \begin{cases} M(t-T,0), & \text{if } T \leq 3 \\ M(t-T,0)e^{-\gamma(T-3)}, & \text{if } T > 3\ . \end{cases}$$

Let ω_s represent the maximum time a cell can spend in storage. Then by integrating over T the total number $\underline{M}(t)$ of cells in storage at time t is found to be:

$$(3.1.7)\quad \underline{M}(t) = \int_0^{\omega_s} M(t,T)dT$$

$$= \int_0^{\omega_s} [(1-F(T)]M(t-T,0)dT\ .$$

The reader will recall from Section 2.2 that O'Fallon defined the distribution of sojourn time of granulocytes in the circulating blood by the probability distribution function

$$K(\tau) = \int_0^{\tau} k(t)dt, \quad \xi > 0\ .$$

The conditional probability that a cell will leave the blood after completing between τ and $(\tau + h)$ units of sojourn time in the blood is given by:

$$(3.1.9) \qquad \frac{\int_{\tau}^{\tau+h} k(t)dt}{1-K(\tau)} \sim \frac{k(\tau)h}{1-K(\tau)} \quad .$$

This represents the fraction of cells of age τ that will leave the blood before they reach age $(\tau + h)$. Let $B(t,\tau)$ represent the number of cells whose sojourn time in the blood is between τ and $(\tau + d\tau)$ at time t. Then,

$$(3.1.10) \qquad B(t,\tau)d\tau \, \frac{k(\tau)h}{1-K(\tau)}$$

cells will leave the blood before they are between $(\tau+h)$ and $(\tau+h+d\tau)$ units old. This results in the equation

$$(3.1.11) \qquad B(t+h, \tau+h)d\tau - B(t,\tau)d\tau = -B(t,\tau)d\tau \, \frac{k(\tau)h}{1-K(\tau)} \quad .$$

$$\text{So,} \qquad \lim_{h\to 0} \frac{1}{h} [B(t+\tau,\tau+h) - B(t,\tau)] = -B(t,\tau) \, \frac{k(\tau)}{1-K(\tau)} \quad .$$

When neutropenia is not in effect, this is a partial differential equation for B whose solution is:

$$(3.1.12) \qquad B(t,\tau) = [1-K(\tau)]B(t-\tau,0) \quad ,$$

where $B(t-\tau,0)$ is the total rate of entry of cells into the blood.

When neutropenia is in effect, the formula for $B(t,\tau)$ depends on the value of t as well as the value of $(t-\tau)$; this is explained in Theorem 3.1.

Theorem 3.1: Let t_s be the time of the start of neutropenia, and let t_n be the time that maximum neutropenia has been achieved in the blood. Assume that neutropenia is obtained by subtracting a fixed percentage wh from each group $(B(t,\tau)d\tau$ during a time interval of length h. Then,

$$B(t,\tau) = \begin{cases} [1-K(\tau)]B(t-\tau,0), & \text{if } t\leq t_s \text{ and } t-\tau\leq t_s \quad \text{(Case 1)} \\ [1-K(\tau)]B(t-\tau,0)e^{-w(t-t_s)}, & \text{if } t_s<t\leq t_n \text{ and } t-\tau\leq t_s \quad \text{(Case 2)} \\ [1-K(\tau)]B(t-\tau,0)e^{-w\tau}, & \text{if } t_s<t\leq t_n \text{ and } t_s<t-\tau\leq t_n \quad \text{(Case 3)} \\ [1-K(\tau)]B(t-\tau,0)e^{-w(t_n-t_s)}, & \text{if } t_n<t \text{ and } t-\tau\leq t_s \quad \text{(Case 4)} \\ [1-K(\tau)]B(t-\tau,0)e^{-w(\tau-t+t_n)}, & \text{if } t_n<t \text{ and } t_s<t-\tau\leq t_n \quad \text{(Case 5)} \\ [1-K(\tau)]B(t-\tau,0), & \text{if } t_n<t \text{ and } t_n<t-\tau \quad \text{(Case 6)} \end{cases}$$

Proof: The proof is based on the following formula which is similar to Equation (3.1.11), but incorporates the effect of neutropenia:

$$(3.1.13) \qquad \lim_{h\to 0} \frac{1}{h}\left[B(t+h,\ \tau+h) - B(t,\tau)\right]$$

$$= \begin{cases} -B(t,\tau)\ \dfrac{k(\tau)}{1-K(\tau)} - wB(t,\tau), & \text{if } t\in[t_s,t_n] \\ -B(t,\tau)\ \dfrac{k(\tau)}{1-K(\tau)}, & \text{if } t\notin[t_s,t_n]\ . \end{cases}$$

The first member of this equation represents the derivative of $B(t,\tau)$ along a line such as the one in Figure 3.1 where $(t-\tau)<t_s$ and $t>t_n$; see Case 4 of the above theorem. Represent the points of this line by $(t-\tau+s,s)$. The Equation (3.1.13) can be written as:

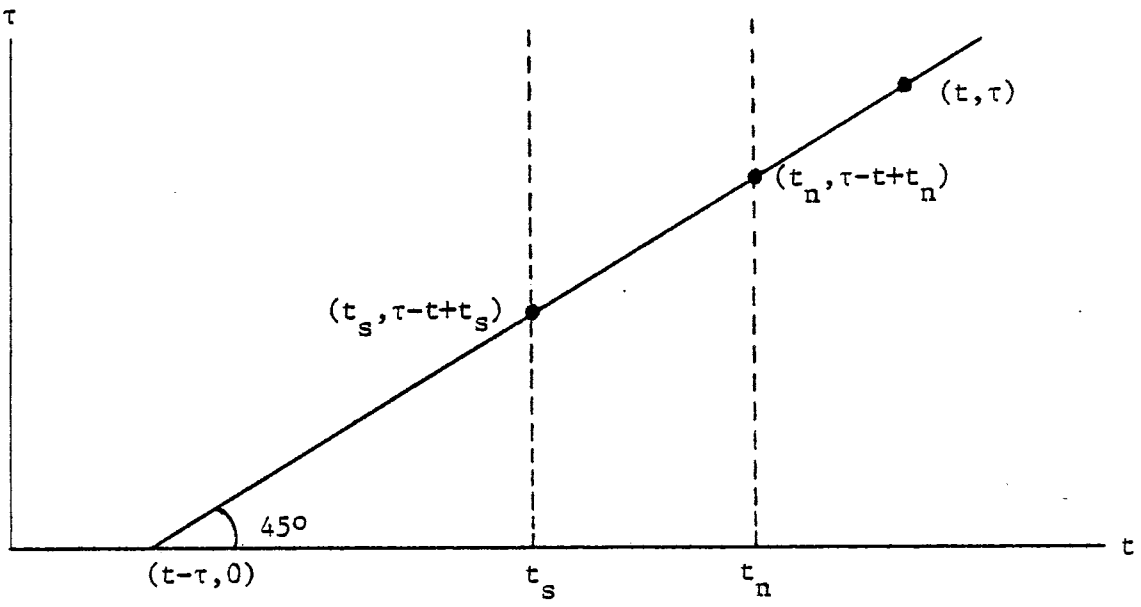

Figure 3.1 Representative diagram of Case 4 of Theorem 3.1

$$\frac{d}{ds} B(t-\tau+s,s)$$

$$= \begin{cases} -B(t-\tau+s,s)\ \frac{k(s)}{1-K(s)} - wB(t-\tau+s,s), & \text{if } s\in[\tau-t+t_s,\tau-t+t_n] \\ -B(t-\tau+s,s)\ \frac{k(s)}{1-K(s)}\ , & \text{if } s\notin[\tau-t+t_s,\ \tau-t+t_n]\ . \end{cases}$$

Now, the theorem expresses $B(t,\tau)$ in terms of $B(t-\tau,0)$. This expression is achieved by first expressing $B(t,\tau)$ in terms of $B(t_n,\tau-t+t_n)$, then expressing $B(t_n,\tau-t+t_n)$ in terms of $B(t_s,\tau-t+t_s)$, and finally expressing $B(t_s,\tau-t+t_s)$ in terms of $B(t-\tau,0)$. Each one of these steps follows from integration of

$$\frac{\frac{d}{ds} B(t-\tau+s,s)}{B(t-\tau+s,s)} = \frac{-k(s)}{1-K(s)} \text{ or } \frac{-k(s)}{1-K(s)} - w\ ,$$

respectively between the relevant values of s. Thus,

$$\log B(t-\tau+s,s)\Big|_{\tau-t+t_n}^{\tau} = \log(1-k(s))\Big|_{\tau-t+t_n}^{\tau},$$

$$\log B(t-\tau+s,s)\Big|_{\tau-t+t_s}^{\tau-t+t_n} = \log(1-k(s))\Big|_{\tau-t+t_s}^{\tau-t+t_n} - w(t_n-t_s),$$

$$\log B(t-\tau+s,s)\Big|_{0}^{\tau-t+t_s} = \log(1-k(s))\Big|_{0}^{\tau-t+t_s}.$$

These results yield

$$B(t,\tau) = B(t_n,\ \tau-t+t_n)\ \frac{1-K(\tau)}{1-K(\tau-t+t_n)}$$

$$B(t_n,\ \tau-t+t_n) = B(t_s,\ \tau-t+t_s)\ \frac{1-K(\tau-t+t_n)e^{-w(t_n-t_s)}}{1-K(\tau-t+t_s)},$$

$$B(t_s,\ \tau-t+t_s) = B(t-\tau,0)\ \frac{1-K(\tau-t+t_s)}{1-K(0)};$$

Compare to Equation (3.1.12). Multiplication of these three results will immediately give the results stated under Case 4 of the theorem. The proofs for the other cases are applications of the same type of argument and can be completed by the reader.

Since $B(t,\tau)$ depends on where t and $(t-\tau)$ are with regard to t_s and t_n, the following result for $\underline{B}(t)$, the total number of cells in the blood, is easily obtained.

Corollary 3.1.1: Assume ω_b is the maximum time a cell can spend in the blood. Under the assumptions of Theorem 3.1,

$$\underline{B}(t) = \int_0^{\omega_b} B(t,\tau)d\tau$$

$$= \begin{cases} \displaystyle\int_0^{\omega_b} [1-K(\tau)]B(t-\tau,0)d\tau, & \text{if } t \leq t_s \\[2ex] \displaystyle\int_{t-t_s}^{\omega_b} [1-K(\tau)]B(t-\tau,0)e^{-w(t-t_s)}d\tau + \int_0^{t-t_s} [1-K(\tau)] \times & \\ \qquad B(t-\tau,0)e^{-w\tau}\,d\tau, & \text{if } t_s \leq t \leq t_n \\[2ex] \displaystyle\int_{t-t_s}^{\omega_b} [1-K(\tau)]B(t-\tau,0)e^{-w(t_n-t_s)}d\tau + \int_{t-t_n}^{t-t_s} [1-K(\tau)] \times & \\ B(t-\tau,0)e^{-w(\tau-t+t_n)}d\tau + \displaystyle\int_0^{t-t_n} 1-K(\tau)B(t-\tau,0)d\tau\,, & \text{if } t_n < t\,. \end{cases}$$

Before going deeper into the study of age distribution and feedback, it seems appropriate to show the relationship between the continuous age distribution model developed above and O'Fallon's continuous model.

Corollary 3.1.2: Let $A(t)\cdot k$ be the number of cells entering storage between times t and t+k. Assume a cell must spend a minimum of q hours in storage. Then,

$$\underline{B}(t) = \int_{-\infty}^{t-q} A(m)dm \int_{q}^{t-m} f(T)e^{-\xi(t-m-T)}\, dT ,$$

where f(T) is defined in Section 2.2.

Proof:

$$\underline{B}(t) = \int_{0}^{\omega_b} B(t,\tau)d\tau, \text{ by Corollary 3.1.1;}$$

$$B(t,\tau) = [1-K(\tau)]B(t-\tau,0), \text{ by Equation (3.1.12)}$$

$$= e^{-\xi\tau}\, B(t-\tau,0)$$

$$= e^{-\xi\tau} \int_{q}^{\omega_s} M(t-\tau,T)\, \frac{f(T)}{1-F(T)}\, dT, \text{ by Equation (3.1.3)}$$

$$= e^{-\xi\tau} \int_{q}^{\omega_s} M(t-\tau-T,0)[1-F(T)]\, \frac{f(T)}{1-F(T)}\, dT ;$$

see the equation following (3.1.4).

Thus,

$$B(t,\tau) = e^{-\xi\tau} \int_{q}^{\omega_s} M(t-\tau-T,0)\, f(T)dT .$$

By the first condition of the corollary $M(t-\tau-T,0) = A(t-\tau-T)$.

So,

$$B(t,\tau) = e^{-\xi\tau} \int_{q}^{\omega_s} A(t-\tau-T)\, f(T)dT .$$

Therefore,

$$\underline{B}(t) = \int_0^{\omega_b} e^{-\xi\tau}\, d\tau \int_q^{\omega_s} A(t-\tau-T)\, f(T)dT\ .$$

In O'Fallon's continuous model both ω_s and ω_b were approximated by ∞. Then by change of variables from T and τ to T and $m = (t-\tau-T)$,

$$\underline{B}(t) = \int_{-\infty}^{t-q} A(m)dm \int_q^{t-m} f(T)e^{-\xi(t-m-T)}\, dT\ .$$

The structure of this expression closely parallels that of O'Fallon's equation for b(t) discussed in Section 2.2. The major difference between the two formulae is that A(m) in our equation is a rate of entry of cells into the storage compartment, whereas in O'Fallon's equation A(m) is the expected specific activity of the DNA-TE of cells entering the storage compartment at time m.

The continuous model developed in this section proved to be useful when no feedback was involved. However, after neutropenia was simulated and the mean time in storage had to be made variable to account for the feedback caused by the decrease of cells in the blood, it was found that the mean time in storage ought to have different values for different age classes. It is almost impossible to simulate the feedback using the continuous model because of the difficulty keeping track of the different mean times in storage for the different age classes. Therefore, the following discrete model was developed.

3.2 Derivation of the Discrete Model

The discrete model derived in this section is patterned after what is called the Leslie or Projection Matrix. The idea incorporated in the Leslie Matrix is presented as a definition in this thesis. The reader is referred to Mathematical Ecology by Pielou (1977) for a detailed study of the matrix. The discrete model will proceed in time intervals of fixed length h.

Let $y(t)$ be the number of cells that enter storage from the mitotic compartment between times t and (t+h). Let $M(t,ih)$ represent the number of cells in storage in the age group ih to (i+1)h at time t. Let PS_i be the probability that a cell in storage of age between ih and (i+1)h at time t will survive, i.e., stay in storage, to time (t+h). Let $B(t,ih)$ represent the number of cells in the blood in the age group ih to (i+1)h at time t. Let PB_i be the probability that a cell in the blood of age between ih and (i+1)h at time t will survive to time (t+h). Then, by definition, the age distribution vector for cells in storage and blood at time (t+h) is given by:

$$\begin{bmatrix} \cdots\cdots \\ M(t+h,i) \\ \cdots\cdots \\ B(t+h,i) \end{bmatrix} =$$

$$\begin{bmatrix} M(t+h,0) \\ M(t+h,h) \\ M(t+h,2h) \\ \cdot \\ \cdot \\ \cdot \\ M(t+h,\alpha h) \\ \hline B(t+h,0) \\ B(t+h,h) \\ B(t+h,2h) \\ \cdot \\ \cdot \\ \cdot \\ B(t+h,\rho h) \end{bmatrix} = \begin{bmatrix} 0 & & & & & 0 & 0 & 0 & \cdot\ \cdot\ \cdot & 0 & 0 \\ PS_0 & & & & & & & & & & \\ & PS_1 & & & & & & & & & \\ & & \cdot & & & & & & & & \\ & & & \cdot & & & & & & & \\ & & & & PS_{\alpha-1} & & & & & & \\ \hline 1-PS_0 & 1-PS_1 & \cdot\ \cdot\ \cdot & 1-PS_{\alpha-1} & & 0 & 0 & \cdot & \cdot & 0 & 0 \\ & & & & & PB_0 & & & & & \\ & & & & & & PB_1 & & & & \\ & & & & & & & \cdot & & & \\ & & & & & & & & \cdot & & \\ & & & & & & & & & PB_{\rho-1} & \end{bmatrix} \begin{bmatrix} M(t,0) \\ M(t,h) \\ M(t,2h) \\ \cdot \\ \cdot \\ \cdot \\ M(t,\alpha h) \\ \hline B(t,0) \\ B(t,h) \\ B(t,2h) \\ \cdot \\ \cdot \\ \cdot \\ B(t,\rho h) \end{bmatrix} + \begin{bmatrix} y(t) \\ 0 \\ 0 \\ \cdot \\ \cdot \\ \cdot \\ 0 \\ \hline 0 \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ 0 \end{bmatrix}$$

Since y(t) is defined to be the number of cells that enter storage from the mitotic compartment between times t and (t+h), then for any time t, y(t) is the youngest component of the age distribution vector; h is fixed at 0.2 of an hour; ih = 0, 0.2, 0.4, ..., 215.8 hours and α = 1079 for the normal distribution of cells in storage; ih = 0, 0.2, 0.4, ..., 269.8 hours and α = 1349 for the exponential sojourn of cells in storage. The maximum time a cell spends in the blood was chosen to be 72 hours; so, the oldest cells in the blood are in age group (71.8 to 72.0) hours and ρ = 359. A detailed study of the matrix and the transition probabilities defined in the matrix will be discussed in great detail in the following chapter.

Before going on to the next chapter the reader should be aware that summing all the age classes of the age distribution vectors results in:

$$(3.1.14) \qquad \underline{M}(t) = \sum_{i=0}^{\alpha} M(t+h,ih);$$

$$(3.1.15) \qquad \underline{B}(t) = \sum_{i=0}^{\rho} B(t+h,ih) \ .$$

4. ANALYSIS OF THE DISCRETE MATHEMATICAL MODEL

4.1 Development of y(t), the Number of Cells That Enter Storage between Times t and (t+h)

According to Cronkite and Vincent (1970) when the Granulocyte Turnover Rate is constant, 6.79×10^7 per hour, the number of cells that enter storage from the mitotic compartment equals 6.79×10^7 per hour. In the discrete model the number of cells in storage and blood is determined every 0.2 of an hour. Thus, in steady state conditions, when Granulocyte Turnover Rate is constant, y(t) was set at 1.358×10^7.

By definition θ is the generation time of a cell; it is the time it takes from a cell's formation until the cell divides. Cronkite and Vincent propose that the bone marrow contains 260×10^7 myelocytes.[1] Assume that when one of these cells divides it enters the storage compartment. Then the number of cells entering storage per hour is expressed by $(260 \times 10^7)/\theta$. Accordingly, the number of cells that enter storage every 0.2 of an hour is

$$y(t) = (260 \times 10^7)/5\theta \ .$$

This thesis acts on the assumption that during feedback θ is changed by one of the following equations:

(4.1.1) $$\theta = 38.291605 + H_2(SC(t-0.2) - 880.67) \times 10^7$$

for the feedback from storage to mitotic compartment,

---

[1] All cell numbers referred to in this chapter are in reality number of cells $\times 10^7$ per kg. of body weight.

(4.1.2) $\theta = 38.291605 + H_2(BL(t-0.2) - 70.68) \times 10^7$

for the feedback from the blood to mitotic compartment.

$SC(t - 0.2)$ represents 10^{-7} x the number of cells in storage at time $(t - 0.2)$. $BL(t - 0.2)$ represents 10^{-7} x the number of cells in the blood at time $(t - 0.2)$. H_2 is measured in hours per cells. The value 70.68×10^7 is the steady state value of cells circulating in the blood. The value 880.68×10^7 is the steady state value of cells in the storage compartment.

4.2 Initial Determination of the Transition Probabilities of Cells in Storage

The reader will recall from Section 2.2 that O'Fallon (1967) defined the distribution of sojourn time in storage by the weighted sum of two probability density functions. This fact is used in expressing the conditional probability that a cell in storage of age between i and $(i + h)$ at time t will survive to time $(t + h)$.

The model for the sojourn in and exit from the storage compartment may be set up in two basically different methods. One method uses the formula (3.1.5) for the conditional probability that a cell will leave storage after spending between T and $(T + k)$ units of time in storage and substitutes $f(t)$ by $\pi f_1(t) + (1 - \pi) f_2(t)$ according to O'Fallon.

The other method considers the cells whose sojourn time is distributed according to the density f_2 (the normal density) and according to f_1 (the exponential density) as two groups, whose life

histories in storage are kept completely separate, one with transition probabilities

$$P_1S = (1 - F_1(T + k))/(1 - F_1(T)),$$

and the other with transition probabilities $P_2S = (1 - F_2(T + k))/(1 - F_2(T))$. According to this method a fraction π of the cells entering storage behaves according to transition probabilities P_1S, and a fraction $(1 - \pi)$ according to transition probabilities P_2S.

It turns out that in steady state the two methods lead to the same overall result (see Appendix 7.9). However, in non-steady state with feedback operating, there is a big difference, and the second method gives results much closer to observations by Patt et al. (1957).

As for the first method, O'Fallon defined the distribution of sojourn in storage by the density $f(t) = \pi f_1(t) + (1 - \pi)f_2(t)$. In conjunction with this, the cumulative distribution function of sojourn time in storage is given by:

$$\int_0^T [\pi f_1(t)dt + (1 - \pi)f_2(t)]dt .$$

The conditional probability that a cell will leave storage after spending between T and T + k units of time in storage given that the cell has spent T units of time in storage is:

$$\frac{\int_T^{T+k} \pi f_1(t) + (1 - \pi)f_2(t)dt}{1 - \pi F_1(T) - (1 - \pi)F_2(T)} .$$

Therefore, with k = 0.2, the conditional probability that a cell will be in storage at age (T + 0.2) given that it has already spent T units of time in storage is

$$(4.2.1) \qquad PS = \frac{1 - \pi F_1(T + 0.2) - (1 - \pi)F_2(T + 0.2)}{1 - \pi F_1(T) - (1 - \pi)F_2(T)} .$$

As for the second method the two transition probabilities P_1S and P_2S are given by the expressions:

$$(4.2.2) \qquad P_1(S) = \frac{1 - F_1(T + 0.2)}{1 - F_1(T)} \qquad \text{and}$$

$$P_2(S) = \frac{1 - F_2(T + 0.2)}{1 - F_2(T)} .$$

In the continuous model the maximum time a cell can spend in the storage or blood is approximated by infinity. However, in reality there must be a maximum finite period of time that a cell can spend in the storage compartment or in the blood. This will be used in the discrete model. Cronkite and Vincent (1970, p. 218) believe that the storage compartment contains the non-dividing myelocytes, the metamyclocytes, and band and segmented neutrophils. They believe there are 270×10^7 metamyelocytes in storage, 360×10^7 band neutrophils in storage, and 250×10^7 segmented neutrophils in storage. They do not postulate the number of non-dividing myelocytes that are in storage. They do believe, however, that the myelocytes spend only about 3 hours in storage before becoming metamyelocytes. Since the non-dividing myelocytes spend such a short time in storage

in this form, this thesis uses 880×10^7 as the total amount of cells in storage when the granulocyte system is in a steady state condition. The steady state condition prevails when the number of cells in storage and blood remains constant, and the rate of entry of cells, or Granulocyte Turnover Rate, into storage and blood is constant.

According to Cronkite and Vincent there are 70×10^7 cells in the blood. They believe the blood is subdivided into two pools--the circulating granulocyte pool which in steady state conditions has 31×10^7 cells, and the marginal granulocyte pool which in steady state conditions has 39×10^7 cells. The total blood granulocyte pool, TBGP, has 70×10^7 cells.

In order to determine P_2S for the normal distribution, computer simulation of Equation (3.1.5) for $\underline{M}(t)$ showed that for $\mu = 129.6$ hours and for the Granulocyte Turnover Rate equal to 6.79×10^7 per hour the storage compartment maintains 880×10^7 cells (see Appendix 7.3 and Table 7.3.2). Further investigation revealed for the continuous model that truncating the maximum time a cell can spend in storage at 216 hours did not yield results significantly different from having the maximum approximated by infinity. Thus, for the normal distribution,

$$(4.2.3) \qquad P_2S = \frac{1 - F(T + 0.2)}{1 - F(T)}$$

$$= \frac{1 - \int_{48}^{T+0.2} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{t - \mu}{\sigma})^2} dt}{1 - \int_{48}^{T} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(t - \mu)^2} dt}$$

(see Appendix 7.7).

In order to determine P_1S for the exponential density function, $f(t) = \gamma e^{-\gamma(t-3)}$ was normalized and truncated to yield a new cumulative distribution function,

$$(4.2.4) \qquad F^*(T) = \frac{1 - e^{-\gamma(T - 3)}}{1 - e^{-\gamma(t^* - 3)}} \quad ,$$

where t^* is the maximum time a cell can spend in storage according to the exponential density function. Computer results show that a value of γ equal to 0.001163 is the only reasonable one that gives 880×10^7 cells in storage in the shortest period of time t^* possible. That is, γ set at .001163 gives 880×10^7 cells in storage if cells spend a maximum of $t^* = 270$ hours in storage (see Appendix 7.3 and Table 7.3.1). Thus, for the exponential density function,

$$(4.2.5) \qquad P_1S = \frac{1 - F^*(T + 0.2)}{1 - F^*(T)}$$

$$= \frac{e^{-\gamma(T + 0.2-3)} - e^{-\gamma(270 - 3)}}{e^{-\gamma(T - 3)} - e^{-\gamma(270 - 3)}}$$

(see Appendix 7.6).

In the above equations the values of μ, σ, and γ are constants in the steady state, but are functions of time t in general. For example in the simulation of neutropenia, the following expressions have.been used with the discrete model:

$$(4.2.6) \qquad \mu = \begin{cases} 129.60 , & \text{if } t < t_n \\ 129.60 + H * (BL(t - 0.2) - 70.68) \times 10^7, & \text{if } t \geq t_n \end{cases}$$

$$\sigma = (\mu - 48)/4.5 ,$$

and

$$(4.2.7) \qquad \gamma = \begin{cases} 0.001163 , & \text{if } t < t_n \\ 0.001163 - G * (BL(t - 0.2) - 70.68), & \text{if } t \geq t_n . \end{cases}$$

$BL(t - 0.2)$ is the same as in Section 4.1. H and G are measured in hours per cells.

4.3 Determination of the Transition Probabilities of Cells in the Blood

Fliedner et al. (1964) seemed to believe that most calls disappear from the blood in between 24.0 and 72.0 hours. In order to determine PB for the distribution of cells in the blood, the density function $k(\tau) = \xi e^{-\xi\tau}$ was normalized and truncated, so that the blood contains approximately 70×10^7 cells when the maximum time a cell is allowed to sojourn in the blood is 72.0 hours. Computer results show that a value of ξ equal 0.096347 gives approximately 70×10^7 cells in the blood (see Appendix 7.3 and Table 7.3.3). Thus,

$$PB = \begin{cases} \dfrac{1 - K^*(\tau + 0.2)}{1 - K(\tau)}, & \text{if } t \notin [t_s, t_n] \\ \dfrac{1 - K^*(\tau + 0.2)}{1 - K(\tau)} - (w * 0.2), & t \in [t_s, t_n], \end{cases}$$

where K* is the new normalized truncated distribution function (see Appendix 7.7)

$$= \begin{cases} \dfrac{e^{-\xi(\tau + 0.2)} - e^{-\xi(72)}}{e^{-\xi(\tau)} - e^{-\xi(72)}}, & \text{if } t \notin [t_s, t_n] \\ \dfrac{e^{-\xi(\tau + 0.2)} - e^{-\xi(72)}}{e^{-\xi(\tau)} - e^{-\xi(72)}} - (w * 0.2), & \text{if } t \in [t_s, t_n]. \end{cases}$$

In the above equation PB depends upon where t is in regard to the simulation of neutropenia. When $t \in [t_s, t_n]$ neutropenia is in effect so that (w*0.2) is subtracted from the transition probability PB of each age group $B(t,\tau)$. When $t > t_n$, no such subtraction occurs but μ, γ and θ are then changed according to the above mentioned feedback formulae. θ is calculated using $H_2 = 2.45$ for the simultaneous feedback and $H_2 = 2.2$ for the cascading feedback mechanism.

4.4 Results of 20 Per Cent Neutropenia Simulation and Further Development of the Model

When simulating 20 per cent neutropenia it is difficult to obtain the first overshoot observed by Patt et al. when using combined transition probabilities.

Since the combined transitional probabilities did not prove helpful, a non-combined approach was used. The non-combined approach was implemented by assuming that of the number of cells entering

storage from the mitotic compartment at time t a certain proportion π will enter storage and have a distribution of sojourn time that is an exponential density function; and a proportion $(1 - \pi)$ will enter storage and have a distribution of sojourn time that is normally distributed. So, of the y(t) cells entering storage at time t, π y(t) will enter the exponential subpopulation; $(1 - \pi)$ y(t) will enter the normal distribution subpopulation. The transition probabilities were calculated separately for each density function, using P_2S for the normal distribution and P_1S for the exponential density function (see Equations (4.2.3) and (4.2.5)).

When μ, σ, and γ are defined as in Section 4.2 coupled with the non-combined feedback, simulation of 20 per cent neutropenia and recovery reveal the first observed overshoot, but it is impossible to obtain the second overshoot that Patt et al. observed approximately 5 hours after neutropenia was completed.

Since it was postulated that the exponential subpopulation exits storage much earlier than the normal distribution subpopulation, it was assumed that cells that leave storage through the normal distribution also respond much slower to the feedback signal from the blood than the cells that exit via the exponential density function. On the basis of this, μ was changed to have a built-in time delay of 5 hours. Thus, instead of Equation (4.2.6),

$$(4.4.1) \qquad \mu = \begin{cases} 129.60 , & t < t_n \\ 129.60 + H * (BL(t - 5) - 70.68) \times 10^7, & t \geq t_n . \end{cases}$$

The built-in time delay for the normal distribution gives a good fit to the graph of Patt et al. (1957). Compare the computer result of 20 per cent neutropenia and recovery of Figure 7.10.1 to the graph of Patt et al., Section 2.2. The parameter values that best fit the data are w = .0363, G = .022, H = .7, and H_2 = 2.2 or 2.450, t_s = 5 hours, and t_n = 5.8 hours.

There is basically no difference between the result in the blood of the two feedback models. Figure 7.10.1 of Appendix 7.10 is a representative graph of the effect that either model has on the number of cells in the blood. The major difference between the two models occurs in the storage compartment. In the simultaneous feedback model the signal is imparted to both the storage and the mitotic compartments by the blood when cells are needed in the blood; this way the storage compartment is maintained at approximately 880 cells at all times. In the non-simultaneous or cascade model, the signal is relayed to the mitotic compartment to release cells to storage only when cells are lost from storage to blood. Thus, the storage compartment is decreased to 865 cells and is increased to its steady state value in about 3½ hours after being depleted. See Figure 7.10.4 of Appendix 7.10 for comparison of the behavior of the storage compartment and comparison of the two models.

4.5 Results of 40 Per Cent Neutropenia and Recovery

It was not easy to extract the behavior of 40 per cent neutropenia and recovery from the graph of Patt et al. depicted in Section 1.4. Thus, the development of 40 per cent neutropenia is somewhat

hypothetical and the parameter values chosen are those that give a quicker recovery of cells in storage. The values of t_s, π, G, H, and H_2 were the same as in 20 per cent neutropenia; t_s = 5.6 hours and μ was simulated using the built-in time delay of 5 hours. See Figure 7.10.2 for results of this simulation. The number of cells in the blood reached an overshoot of 72 cells at 0.2 hours after neutropenia was achieved, and reached a second overshoot of 73 cells about 5 hours after neutropenia was achieved.

The effect of the large value of G depletes the storage compartment to 847 cells and by time 12 hours the storage compartment has decreased to about 788 cells. Because of the depletion of the storage compartment G was changed to 0.0120. With this new value of G, the blood reaches only one overshoot, and this occurs about 5 hours after achievement of neutropenia. The storage compartment is depleted to 836, but in 0.2 of an hour is in excess of 880 cells. See Figure 7.10.2 of Appendix 7.10 for representative graph of 40 per cent neutropenia using G is 0.0012. See Figure 7.10.5 for comparison of the behavior of the storage with G is 0.0120 and G is 0.022.

4.6 Discussion of Various Discrete Models Tested and Found Unacceptable

Before the time delay model of 5 hours was accepted as the model that best fits the data of Patt *et al.* (1957), various other models were developed and tested against their data. Figure 1.4.1 represents that data obtained by Patt *et al.* in graphical form.

The first model tested achieved 20 per cent neutropenia in the blood in the same manner as the time delay model of 5 hours. The

blood was then allowed to recover without instituting a feedback mechanism in the model. Figure 7.10.7 of Appendix 7.10 is representative of neutropenia and recovery for either the normal distribution or the exponential density in storage. It is apparent that the number of cells in the blood will recover without feedback, but recovery is quite slow. It is impossible to obtain any of the overshoots obtained by Patt et al. using this model.

The second model tested used the combined probability density function of cells in storage. The feedback was introduced using the formulae for μ, σ and γ given by Equations (4.2.6) and (4.2.7). Figure 7.10.8 of Appendix 7.10 shows that when $\pi = 0.05$, H is given approximately the largest value possible, and G is increased from 0.00001 to 0.001; the first overshoot of cells in the blood obtained by Patt et al. around 0.2 of an hour after achievement of neutropenia is still not reached. To determine if it is possible to reach this first overshoot obtained by Patt et al., two programs were run each letting the exponential part of the combined probability density function, $F_1(t)$, be approximately 1, after achievement of neutropenia. One program was modelled with $\pi = 0.05$ and $H = 5.0$. The other program was modelled with $\pi = 0.40$ and $H = 5.0$ (see Figure 7.10.9 of Appendix 7.10). The first overshoot seen by Patt et al. shortly after the achievement of 20 per cent neutropenia in the blood is not obtained by either $\pi = 0.05$ or $\pi = 0.40$. Since H has approximately the maximum possible value and $F_1(t)$ is approximately 1, it appears that the combined probability density of cells in storage is not the best way to model the system.

The third model kept the life histories of the density functions in storage separate as described in Section 4.4. Again μ, σ, and γ are obtained by formulae (4.2.6) and (4.2.7). The feedback in this model was first attempted by letting $\gamma = 0.001163$ and letting μ vary according to the number of cells in the blood. This was done by letting $G = 0.0$ and $H > 0$ (see Figure 7.10.10 of Appendix 7.10). Using this model, it is possible to obtain the first overshoot of cells in the blood shortly after attainment of 20 per cent neutropenia. However, the second overshoot that Patt et al. obtained about 5 hours after achievement of neutropenia was not obtained.

This third model was then modified to allow γ to vary with the number of cells in the blood. This was accomplished by letting $G > 0$ and $H > 0$. See Figure 7.10.11 of Appendix 7.10. Again it is possible to reach the first overshoot, but the second overshoot of cells in the blood after achievement of 20 per cent neutropenia was not obtained. This third model was then changed to allow ξ to vary when the mean time in storage is variable. This was accomplished by letting

$$\xi = \begin{cases} 0.096347 \ , & \text{if } t \leq t_n \\ 0.096347 - G_1(BL(t-0.2) - 70.680) \ , & \text{if } t > t_n \end{cases}$$

for cells with the exponential density in storage, and

$$\xi = \begin{cases} 0.096347 \ , & \text{if } t \leq t_n \\ 0.096347 - H_3(BL(t-0.2) - 70.68) \ , & \text{if } t > t_n \end{cases}$$

for cells with the normal density in storage.

Varying the average time a cell spends in the blood did not significantly alter the result of the third model (see Figure 7.10.12 of Appendix 7.10). Again it is possible to reach the first overshoot after attainment of neutropenia in the blood, but not the second overshoot.

5. PLANS FOR FUTURE STUDY

5.1 Modification of y(t)

For the storage and blood compartments, age distribution vectors and the Leslie Matrix were used to determine the number of cells in the blood and storage at time t. In order for the mathematical model to be consistent the age distribution study similar to the one used for storage and blood should be developed for the mitotic compartment. In order to do this the mitotic compartment needs to be partitioned into at least four compartments: stem cell, myeloblast, promyelocyte, and dividing myelocyte compartments. Generation time is needed for cells in each compartment, as well as the time each type of cell spends in the four subphases. In addition, the distribution of sojourn time of cells in each compartment is needed. This model will then generate a more realistic y(t).

5.2 Extension of the Two Types of Models

Up until this time the two models studied are dependencies of the storage compartment on the number of cells in the blood and dependency of the mitotic compartment on storage, compared to dependency of both the mitotic and storage compartments on the number of cells in the blood. After compartmentalizing the mitotic compartment these two models can be extended to include the mitotic compartment in two ways. One way is to have storage dependent upon the number of cells in the blood and to have each mitotic compartment dependent upon the next generation of cells in progression of maturity. A second model would be to have all compartments in the mitotic

compartment, as well as the storage compartment, dependent upon the number of cells in the blood.

5.3 Introduction of Tracer

The reader will recall from Section 1.1 that the present study is to serve as a pilot one for stimulating a feedback model without the involvement of tracer. The final parameter values will depend on the best fit of the model to the orthoradiophosphate data on the horse obtained by Dr. Walker.[1] This means that the present feedback studies will have to be changed to incorporate the effect of tracer into the mathematical equations. The feedback mechanism will still have to depend on the number of cells in the blood, but the orthoradiophosphate study will involve labelled cells that enter the blood from storage being dependent upon the total number of cells in the blood.

5.4 Neutropenias in Excess of 50 Per Cent

In our present study, for neutropenias up to 50 per cent the minimum obligatory time in storage for the normal distribution is 48 hours, and for the exponential density the minimum obligatory time in storage is 3 hours. Patt et al. (1957) seem to suggest that for neutropenias in excess of 50 per cent the minimum transit time in storage is greatly reduced and the proportion of band neutrophils to segmented is greatly increased in the blood. Thus, in simulating neutropenias in excess of 50 per cent the minimum transit times in storage will be reduced to see the simulated effect of these changes on the number of granulocytes in the blood.

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[1] See Walker et al. (1973).

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7. APPENDICES

7.1 Normalization of the Exponential Density Function for Storage

The reader will recall from Section 2.2 that O'Fallon (1967) defined the exponential density function for cells in storage by $f(t) = \gamma e^{-\gamma(t-3)}$. Now, let $f^*(t)$ be the normalized form of $f(t)$. Then,

$$f^*(t) = \frac{f(t)}{\int_3^{t^*} f(t)dt}$$

where t^* is the maximum time a cell can spend in storage; i.e., $t^* = 270$. So,

$$f^*(t) = \frac{\gamma e^{-\gamma(t-3)}}{\int_3^{t^*} \gamma e^{-\gamma(t-3)}\, dt}$$

$$= \frac{\gamma e^{-\gamma(t-3)}}{1 - e^{-\gamma(270-3)}} .$$

So, the corresponding normalized cumulative distribution function is

$$F^*(T) = \int_3^T \frac{\gamma e^{-\gamma(t-3)}}{1 - e^{-\gamma(270-3)}}\, dt$$

$$= \frac{1 - e^{-\gamma(T-3)}}{1 - e^{-\gamma(270-3)}} .$$

7.2 Normalization of the Distribution Function for the Blood

It was mentioned in Section 2.2 that O'Fallon (1967) defined the exponential density function for the blood by

$$k(t) = \xi e^{-\xi t} .$$

Let $k^*(t)$ be the normalized form of $k(t)$. Then,

$$k^*(t) = \frac{k(t)}{\int_0^{t^*} k(t)dt}$$

where t^* is the maximum time a cell can spend in the blood, with $t^* = 72$. So,

$$k^*(t) = \frac{\xi e^{-\xi t}}{\int_0^{72} \xi e^{-\xi t}\, dt}$$

$$= \frac{\xi e^{-\xi t}}{1 - e^{-72t}} .$$

So, the corresponding normalized cumulative distribution function is

$$K^*(\tau) = \int_0^{\tau} \frac{\xi e^{-\xi t}}{1 - e^{-72t}}\, dt$$

$$= \frac{1 - e^{-\xi \tau}}{1 - e^{-72\xi}} .$$

7.3 Proof of Constancy of Cells in Blood and Storage When GTR Is Constant

Theorem 7.3.1: Assume the rate of entry of cells into storage is constant; then the number of cells in storage that have an exponential sojourn density is constant. When this rate of entry is 6.79 x 10^7 per hour per kg. of body weight and $\gamma = 0.00163$, this constant number in storage is 880 x 10^7 per kg. of body weight.

Proof: By Equation (3.1.7):

$$\underline{M}(t) = \int_0^{\omega_s} M(t-T,0)[1-F(T)]dT$$

$$= 3(6.79) + \int_3^{\omega_s} M(t-T,0)\left[\frac{e^{-\gamma(T-3)} - e^{-267\gamma}}{1 - e^{-267\gamma}}\right]dT$$

$$= 3(6.79) + \frac{6.79}{1 - e^{-267\gamma}}\left[\int_3^{270} e^{-\gamma(T-3)} - e^{-267\gamma}\right]dT$$

$$= 20.37 + \frac{6.79}{1 - e^{-267\gamma}}\begin{bmatrix}\frac{1}{\gamma}(1 - e^{-267\gamma}) \\ -e^{-267\gamma}(267)\end{bmatrix}$$

When $\gamma = 0.001163$ and $e^{-267\gamma} = 0.733$

$$\underline{M}(t) = 20.37 + 25.43[229.50 - 195.70]$$

$$\sim 880 .$$

Theorem 7.3.2: Assume the rate of entry of cells into storage is constant; then the number of cells in storage is constant that have a normal distribution of sojourn in storage. When this rate of entry is 6.79×10^7 per hour per kg. of body weight and $\mu = 129.60$, this constant number in storage is 880×10^7 per hour per kg. of body weight.

Proof: Approximating ω_s by ∞:

$$\underline{M}(t) = \int_0^{\omega_s} M(t-T,0)[1-F(T)]dT$$

$$\sim 48(6.79) + \int_{48}^{\infty} M(t-T,0)[1-F(T)]dT$$

$$= 48(6.79) + 6.79 \int_{48}^{\infty} dT \int_{T}^{\infty} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{t-u}{\sigma})^2} dt .$$

Let $dV = dT$ and $V = T - 48$,

$$u = \int_{T}^{\infty} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{t-u}{\sigma})^2} dt$$

and $$\frac{du}{dT} = -\frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{T-u}{\sigma})^2} .$$

Then $\underline{M}(t)$ becomes

$$48(6.79) + (6.79) \int_{48}^{\infty} u(T)V'(T)dT$$

$$= 48(6.79) + 6.79 \left((T-48) \int_{T}^{\infty} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{T-\mu}{\sigma})^2} \right)_{48}^{\infty}$$

$$+ 6.79 \int_{48}^{\infty} (T-48) \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{T-\mu}{\sigma})^2} dT$$

$$= 48(6.79) + 6.79 \int_{48}^{\infty} (T-48) \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{T-\mu}{\sigma})^2} dT .$$

Let $T = \mu + \sigma y$ and $y = \frac{T-\mu}{\sigma}$.

Then,

$$\underline{M}(t) = 48(6.79) + 6.79 \int_{\frac{48-u}{\sigma}}^{\infty} (\mu + \sigma y - 48) e^{-\frac{1}{2}y^2} \frac{dy}{\sqrt{2\pi}}$$

$$= 48(6.79) + 6.79 \left[(\mu-48) \int_{\frac{48-u}{\sigma}}^{\infty} e^{-\frac{1}{2}y^2} \frac{dy}{\sqrt{2\pi}} + \sigma \int_{\frac{48-\mu}{\sigma}}^{\infty} ye^{-\frac{1}{2}y^2} \frac{dy}{\sqrt{2\pi}} \right]$$

$$= 48(6.79) + 6.79\left[(\mu-48)\left[1 - F\left(\frac{48-\mu}{\sigma}\right)\right] + \frac{\sigma}{\sqrt{2\pi}}\int_{\frac{48-\mu}{\sigma}}^{\infty} ye^{-\frac{1}{2}y^2}\,dy\right]$$

$$= 48(6.79) + 6.79\left[(\mu-48)\left(1 - F\left(\frac{48-\mu}{\sigma}\right)\right) - \frac{\sigma}{\sqrt{2\pi}}\, e^{-\frac{1}{2}y^2}\Big|_{\frac{48-\mu}{\sigma}}^{\infty}\right]$$

When $\mu = 129.6$ and $\sigma = 18.133$

$$\underline{M}(t) \sim 48(6.79) + 6.79\left[(129.60 - 48)\left(1 - F\left(\frac{-81.6}{18.133}\right)\right)\right]$$

$$\sim 48(6.79) + 6.79(8.16)$$

$$\sim 880\ .$$

Theorem 7.3.3: Assume the rate of entry of cells into storage is constant; then the number of cells in the blood is constant. When this constant rate of entry is 6.79×10^7 per hour per kg. of body weight and $\xi = 0.096347$, this constant number in the blood is 70×10^7 per kg. of body weight.

Proof: From the proof of Corollary 3.1.2 we borrow:

$$\underline{B}(t) = A \int_0^{\omega_b} e^{-\xi\tau}\, d\tau \int_q^{\omega_s} f(T)dT ,$$

where A is the constant rate of entry into storage. So,

$$\underline{B}(t) = \frac{A}{\xi}\,(1 - e^{-\xi\omega_b})(F(\omega_s) - F(q))$$

$$- \frac{A}{\xi}\,(1 - e^{-\xi\omega_b})(F(\infty) - F(q))$$

$$= \frac{A}{\xi}\,(1 - e^{-72\xi}) .$$

With A = 6.79 and ξ = 0.096347, this expression is equal to

$$\frac{6.79}{0.096347}\,(1 - e^{-6.936984})$$

$$= 70.47\ (1 - .00097)$$

$$\sim 70 .$$

$\underline{M}(t)$ was calculated on the computer for various values of γ for the exponential density and various values of μ and σ for the normal distribution. $\underline{B}(t)$ was calculated for various values of ξ. The following tables show the relationship of γ and μ to the total number of cells in storage and of ξ to the total number of cells to the blood.

Table 7.3.1 Shows as γ increases the total number of cells in storage decreases with exponential density; values obtained from continuous model

γ	Total Number of Cells in Storage x 10^7
0.001163	879.997
0.101163	87.489
0.201163	54.1237
0.301163	42.915
0.401163	37.296
0.501163	33.9184
0.601163	31.45
0.701163	30.05
0.801163	28.845
0.9011628	27.904
	t* = 270 hours
0.0001	743.2483
0.11001	82.09167
0.21001	52.70
0.31001	43.00
0.41001	36.93
0.51001	33.68
	t* = 216 hours

Table 7.3.2 Shows as μ and σ decrease the total number of cells in storage decreases; values obtained from continuous model

μ	σ	Total Number of Cells in Storage $\times 10^7$
129.60	18.133	879.985
119.60	15.91	812.08
109.60	13.689	744.1854
99.60	11.466	676.285
89.60	9.244	608.385
79.60	7.022	540.44
69.60	4.80	472.585
		$t^* = 216$ hours

Table 7.3.3 Shows as ξ increases the total number of cells in blood decreases; values obtained from continuous model

ξ	Total Number of Cells in Blood x 10^7
0.096347	70.03
0.196347	34.60
0.296347	22.916
0.396347	17.133
0.496347	13.68
0.596347	11.38
0.696347	9.75
0.796347	8.52
0.896347	7.57
0.996347	6.81
	t^* = 72.0 hours

7.4 Calculation of the Average Time a Cell Spends in Storage

The average time a cell spends in storage with normalized truncated exponential density is

$$= \frac{\int_3^{\omega_s} tf^*(t)dt}{\int_3^{\omega_s} f^*(t)dt}$$

$$= \frac{\int_3^{\omega_s} t\frac{\gamma e^{-\gamma(t-3)}}{1-e^{-267\gamma}}\,dt}{\int_3^{\omega_s} \frac{\gamma e^{-\gamma(t-3)}}{1-e^{-267\gamma}}\,dt}$$

$$= \frac{\int_3^{\omega_s} t\, e^{-\gamma(t-3)}\,dt}{\int_3^{\omega_s} e^{-\gamma(t-3)}\,dt} \quad .$$

Integration by parts yields

$$= \frac{-\frac{\omega_s}{\gamma} e^{-\gamma(\omega_s-3)} + \frac{3}{\gamma} + \frac{1}{\gamma^2}\left[1 - e^{-\gamma(\omega_s-3)}\right]}{\frac{1}{\gamma}\left[1 - e^{-\gamma(\omega_s-3)}\right]} \quad .$$

When $\gamma = 0.01163$ and $\omega_s = 270$ the expression

$$= \frac{-270(.733) + 3 + \frac{1}{0.001163}(.267)}{.267}$$

$$\sim 121.61 \text{ hours.}$$

For the normal density (not truncated or normalized) the average sojourn time in storage is simply

$$48 + \mu = 48 + 129.6 = 177.6 \ .$$

7.5 Calculation of the Average Time a Cell Spends in the Blood

The average time a cell spends in the blood

$$= \frac{\int_0^{\omega_b} tk(t)\,dt}{\int_0^{\omega_b} k(t)\,dt}$$

$$= \frac{\int_0^{\omega_b} t \dfrac{\xi e^{-\xi t}}{1 - e^{-72\xi}}\,dt}{\int_0^{\omega_b} \dfrac{\xi e^{-\xi t}}{1 - e^{-72\xi}}\,dt}$$

$$= \frac{\int_0^{\omega_b} t\, e^{-\xi t}\,dt}{\int_0^{\omega_b} e^{-\xi t}\,dt} \ .$$

Integration by parts yields

$$= \frac{-\frac{\omega_b}{\xi} e^{-\xi\omega_b} + \frac{1}{\xi^2}\left[1 - e^{-\xi\omega_b}\right]}{\frac{1}{\xi}\left[1 - e^{-\xi\omega_b}\right]} \ .$$

When $\xi = 0.096347$ and $\omega_b = 72$ the expression

$$= \frac{-747.298(.00097) + (107.772)(.99903)}{10.369082}$$

$$\sim 10.314 \text{ hours.}$$

7.6 Calculation of P_1S for the Exponential Density in Storage

The conditional probability that between sojourn time t_1 and time t_2 a cell leaves the storage compartment, given that at sojourn time t_1 the cell is in this compartment, is

$$\frac{F(t_2) - F(t_1)}{1 - F(t_1)} .$$

The conditional probability that at sojourn time t_2 a cell is in the compartment, given that it was there at sojourn time t_1, is

$$\frac{1 - F(t_2)}{1 - F(t_1)} .$$

Thus, for the exponential density in storage

$$P_1S = \frac{1 - \frac{e^{-\gamma(t_2-3)}}{e^{-\gamma(270-3)}}}{1 - \frac{e^{-\gamma(t_1-3)}}{e^{-\gamma(270-3)}}}$$

$$= \frac{e^{-270\gamma} - e^{-\gamma t_2}}{e^{-270\gamma} - e^{-\gamma t_1}} .$$

7.7 Calculation of P_2S for the Normal Distribution in Storage

Using the same argument as in Section 7.6,

$$P_2S = \frac{1 - F(t_2)}{1 - F(t_1)} .$$

where

$$F(T) = \int_{48}^{T} f(t)dT,$$

and with $f(t)$ the density of the normal distribution with mean μ and variance σ^2.

Thus,

$$P_2S = \frac{\int_{t_2}^{\infty} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{t-\mu}{\sigma})^2} dt}{\int_{t_1}^{\infty} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{t-\mu}{\sigma})^2} dt} .$$

7.8 Calculation of PB for the Blood

The conditional probability that between sojourn t_1 and sojourn time t_2 a cell leaves the blood, given that at sojourn time t_1 the cell is in this compartment, is

$$\frac{K(t_2) - K(t_1)}{1 - K(t_1)} .$$

The conditional probability that at sojourn time t_2 a cell is still in the blood, given that it was there at t_1, is

$$\frac{1 - K(t_2)}{1 - K(t_1)} \quad .$$

Thus,

$$PB = \frac{1 - \frac{e^{-\xi(t_2)}}{e^{-\xi(72)}}}{1 - \frac{e^{-\xi(t_1)}}{e^{-\xi(72)}}}$$

$$= \frac{e^{-\xi(72)} - e^{-\xi(t_2)}}{e^{-\xi(72)} - e^{-\xi(t_1)}} \quad .$$

7.9 Proof That in Steady State the Results of the Combined Transition Probabilities in the Leslie Matrix Are the Same as the Results of Separating the Histories of the Two Distributions in Storage

Assume that the average time that a cell spends in storage is constant. Let t_o stand for the time the cells enter storage. Then, in the case of two separate pipelines or histories the number of cells surviving in storage to time $t_o + h$ is

$$\frac{1 - F_j(t_o + h)}{1 - F_j(t_o)} \; y(t_o) \; ,$$

where $F_j\ (t_o + h)$ is the cumulative distribution function of sojourn time in "pipeline" j at $t_o + h$; $j = 1,2$. The number of cells surviving to time $t_o + 2h$ is

$$\frac{1 - F_j(t_o + 2h)}{1 - F_j(t_o + h)} \cdot \frac{1 - F_j(t_o + h)}{1 - F_j(t_o)} \; y(t_o)$$

$$= \frac{1 - F_j(t_o + 2h)}{1 - F_j(t_o)} \; y(t_o); \quad j = 1,2 \; .$$

Proceeding in this manner, the number of cells in storage surviving to time $t_o + ih$ is

$$\frac{1 - F_j(t_o + ih)}{1 - F_j(t_o + ih - h)} \cdots \frac{1 - F_j(t_o + 2h)}{1 - F_j(t_o + h)} \cdot \frac{1 - F_j(t_o + h)}{1 - F_j(t_o)} \; y(t_o)$$

$$= \frac{1 - F_j(t_o + ih)}{1 - F_j(t_o)} \; y(t_o)$$

$$= \frac{1 - F_j(t_o + ih)}{1} \; y(t_o)$$

since t_o is less than the minimum obligatory time required in storage, so that $F(t_o) = 0$.

Combining these results, the model of two separate pipelines gives the surviving in storage to time $t_o + ih$ as

$$\pi[1 - F_1(t_o+ih)]y(t_o) + (1 - \pi)[1 - F_2(t_o+ih)]y(t_o)$$

$$(7.9.1) \qquad = [1 - \pi F_1(t_o+ih) - (1 - \pi)F_2(t_o+ih)]y(t_o) \; .$$

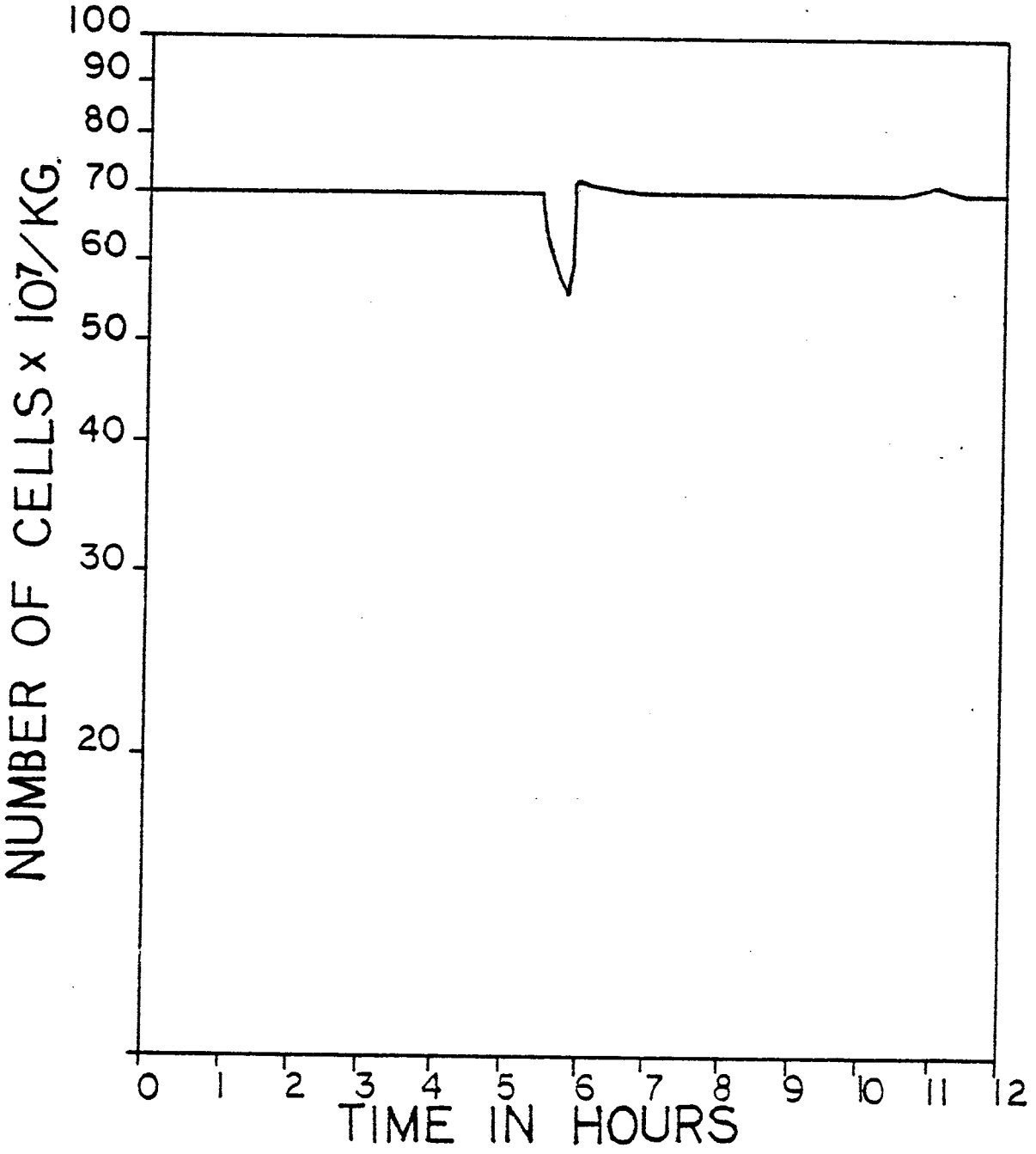
Using the same procedure for the combined probability as in the case of separate pipelines, the number of cells surviving in storage to time $t_o + ih$ is

$$\left[\frac{1 - \pi F_1(t_o+ih) - (1 - \pi)F_2(t_o+ih)}{1 - \pi F_1(t_o+ih-h) - (1-\pi)F_2(t_o+ih-h)}\right]$$

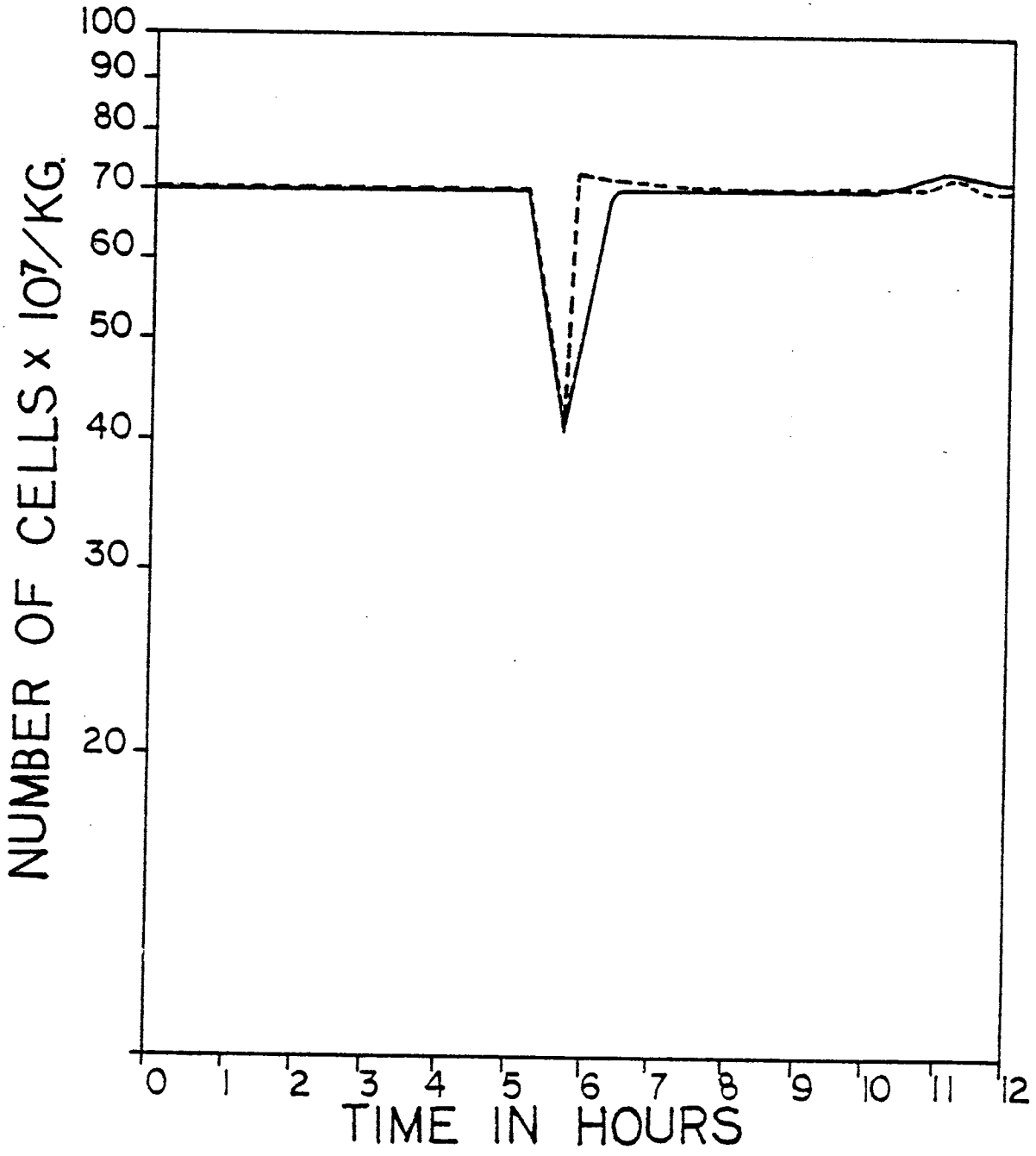
$$x \cdots x \left[\frac{1 - \pi F_1(t_o+2h) - (1-\pi)F_2(t_o+2h)}{1 - \pi F_1(t_o+h) - (1-\pi)F_2(t_o+h)}\right]$$

$$x \left[\frac{1 - \pi F_1(t_o+h) - (1-\pi)F_2(t_o+h)}{1 - \pi F_1(t_o) - (1-\pi)F_2(t_o)}\right] y(t_o) ,$$

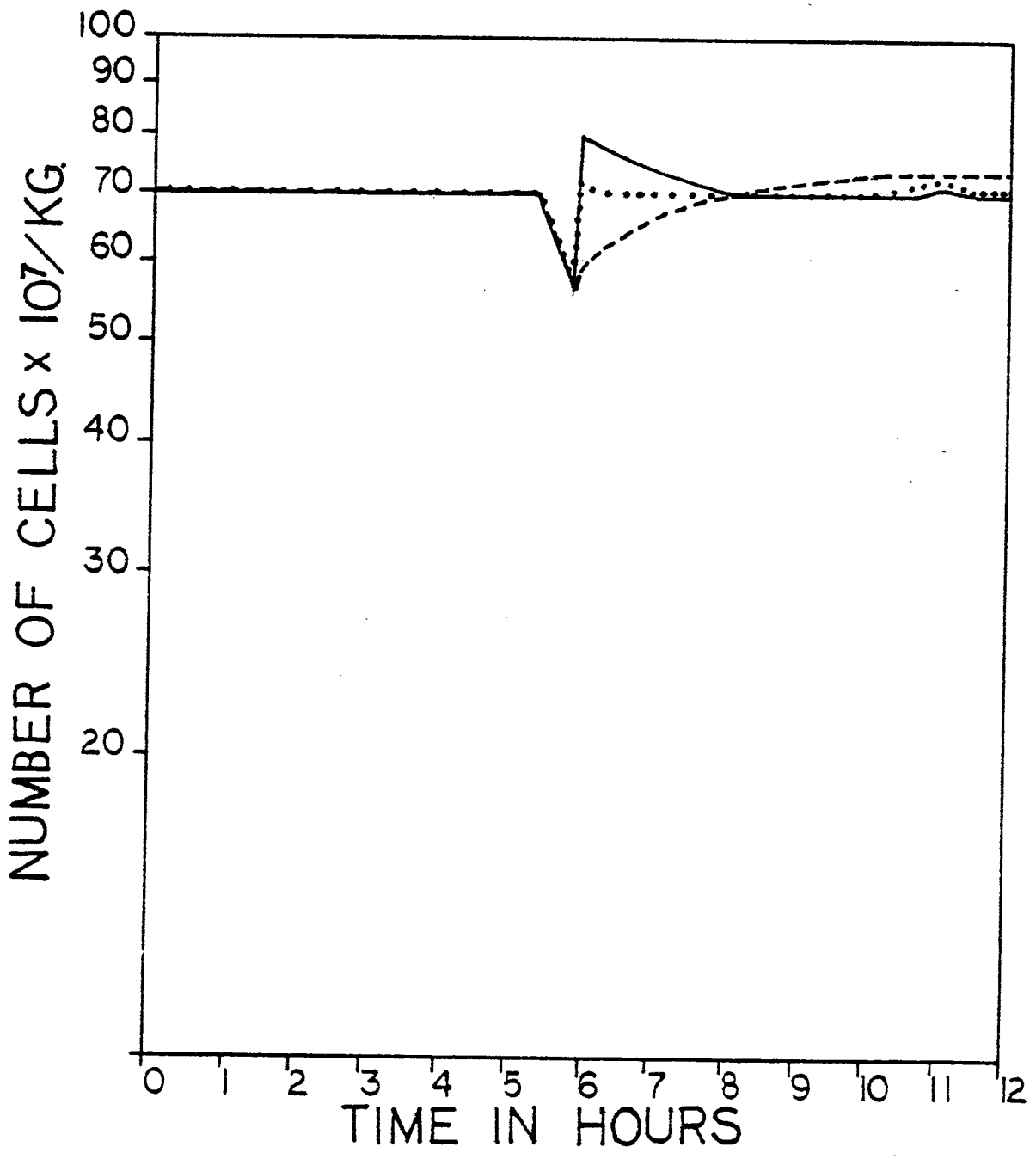
which expression is the same as in Equation (7.9.1).



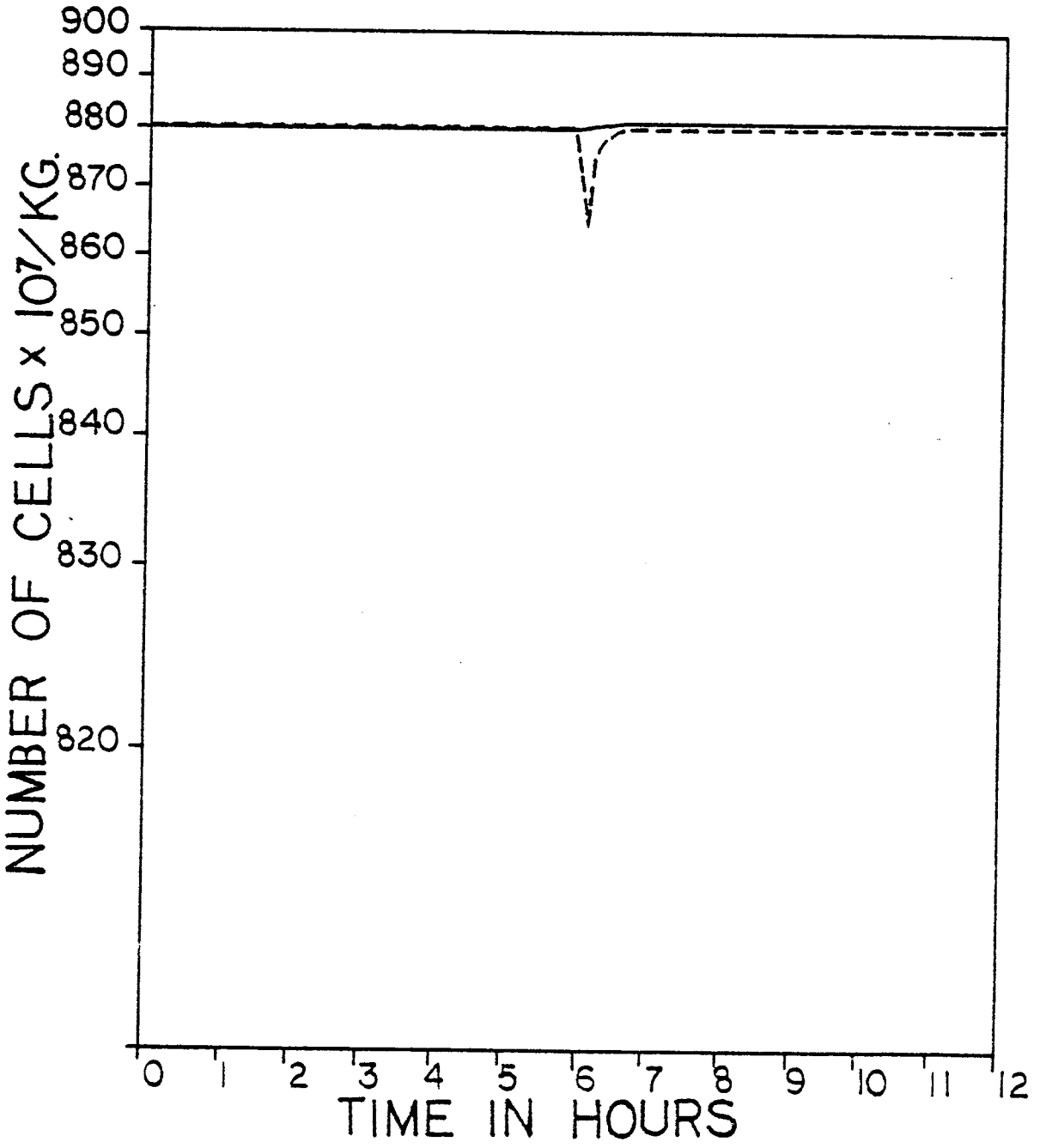
π 0.30
G 0.022
H 0.70
H_2 2.20/2.45

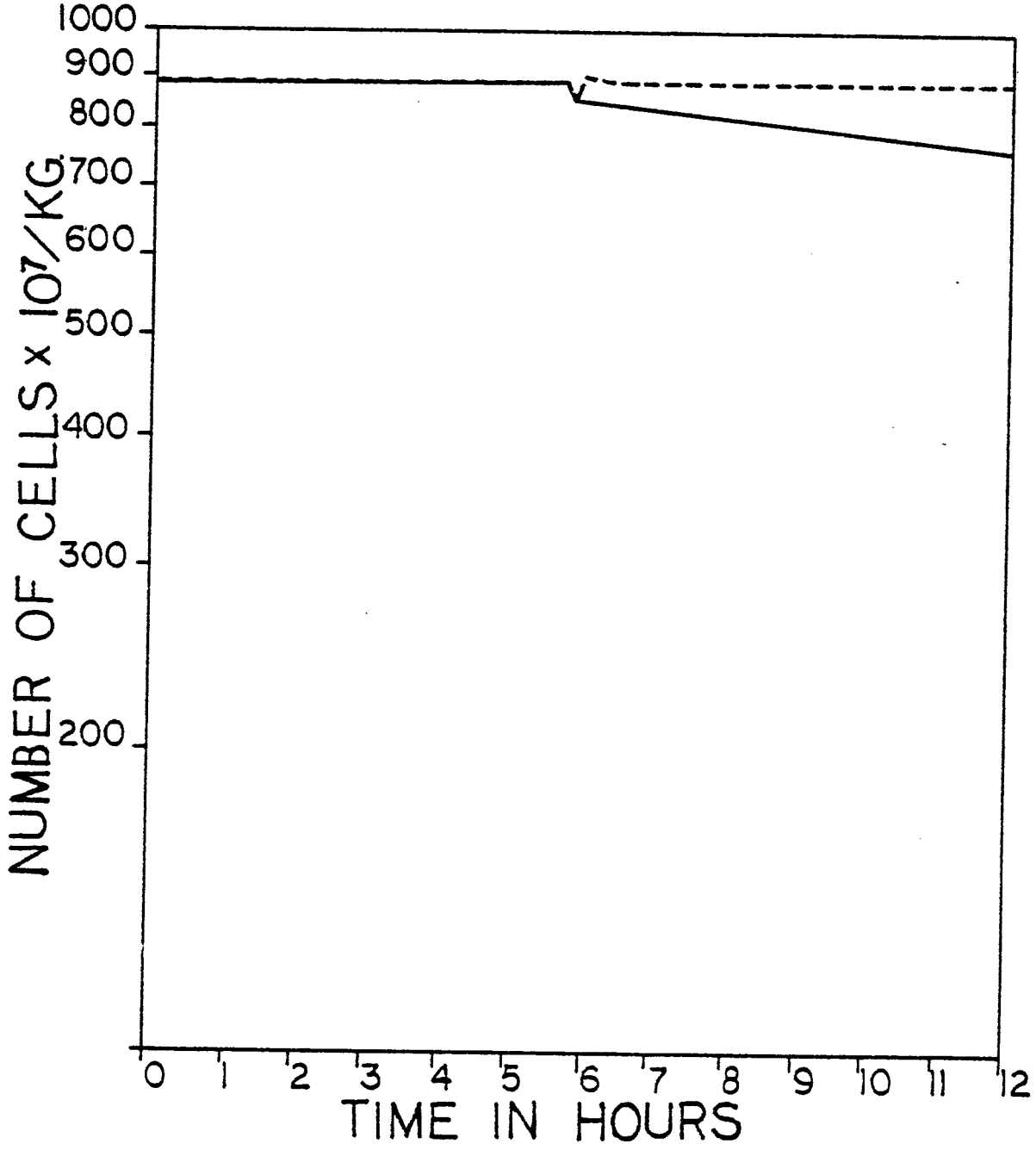


—	--
π 0.30	π 0.30
G 0.012	G 0.22
H 0.70	H 0.70
H_2 2.20/1.28	H_2 2.20/2.45

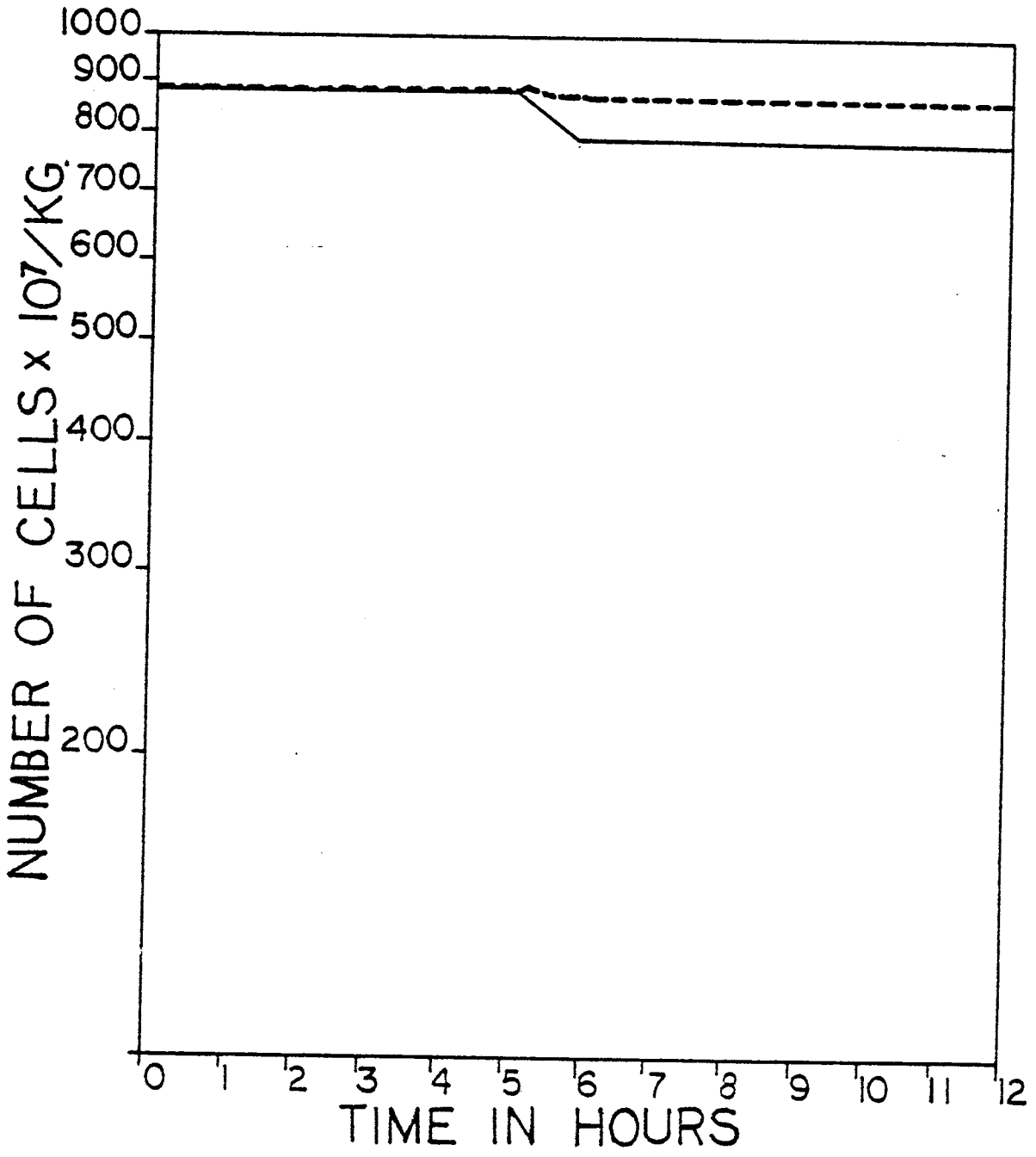


····	--	—
π 0.45	π 0.05	π 0.45
G 0.0150	G 0.022	G 0.022
H 0.70	H 0.90	H 0.50
H_2 2.20	H_2 2.20	H_2 2.20

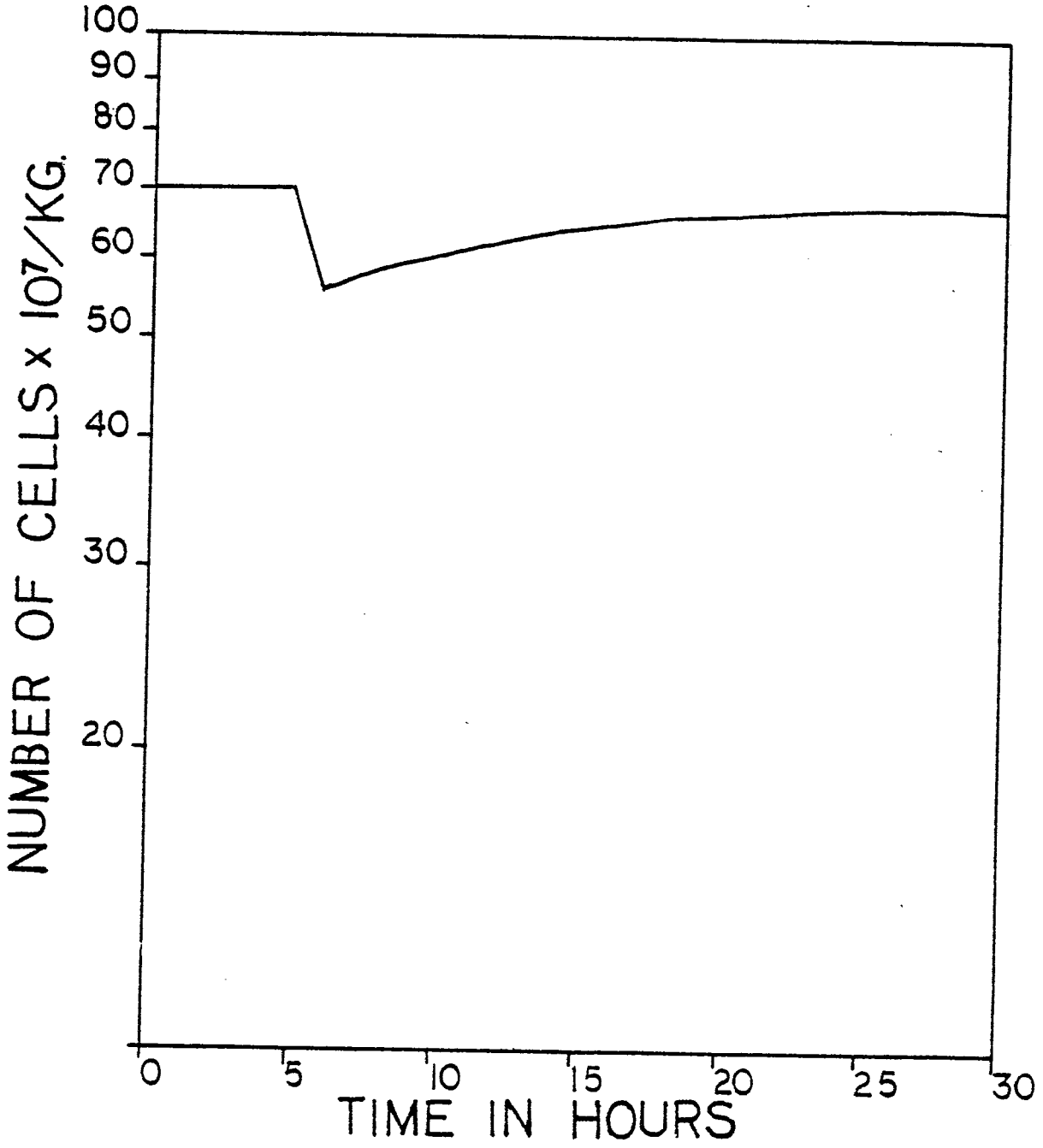

900
890
880
870
860
850
840
830
820
NUMBER OF CELLS x 10⁷/KG.
0
1
2
3
4
5
6
7
8
9
10
11
12
TIME IN HOURS
SIMULTANEOUS FEEDBACK
CASCADING OF FEEDBACK

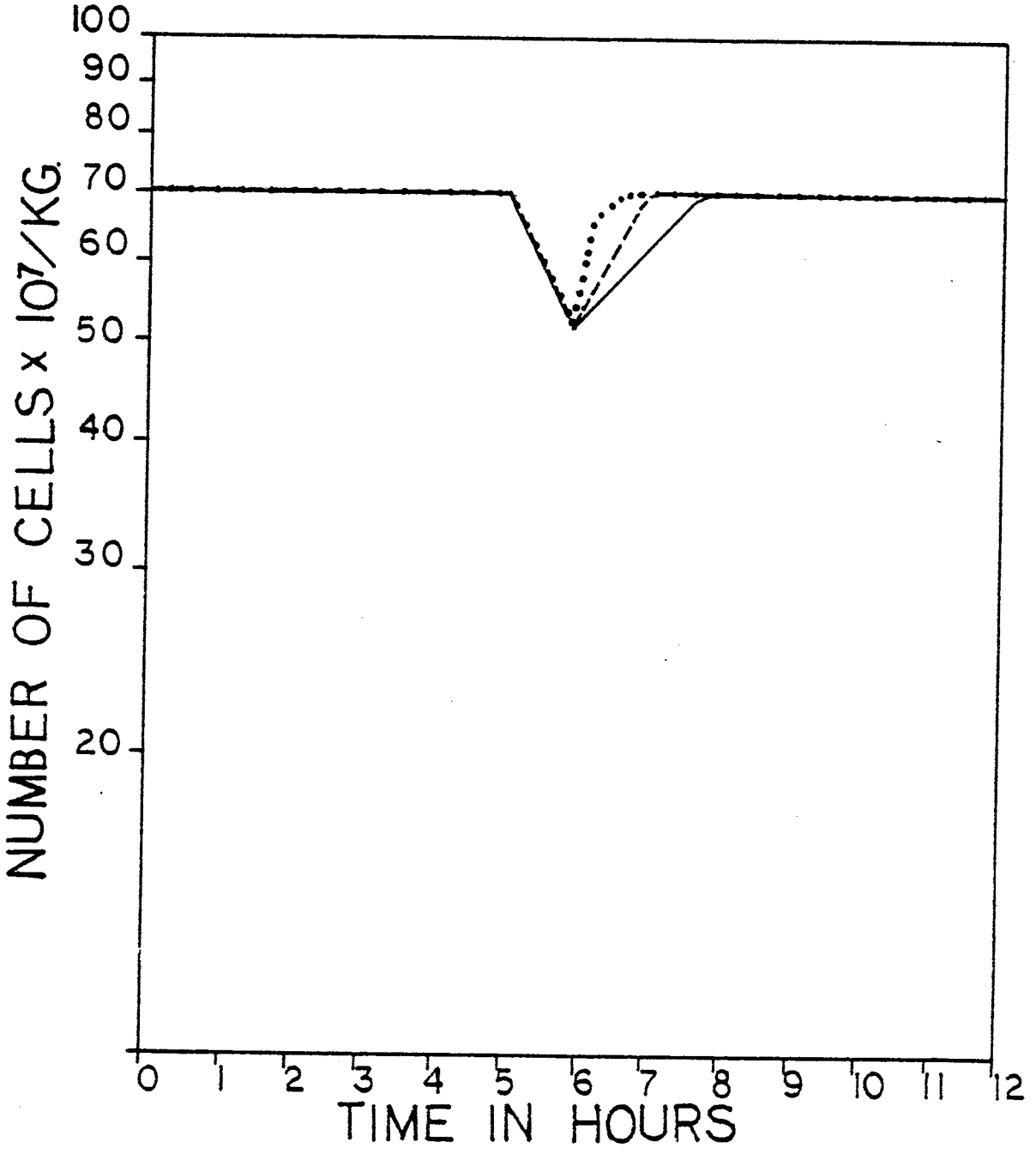


—	--
π 0.30	π 0.30
G 0.022	G 0.012
H 0.70	H 0.70
H_2 2.20	H_2 2.20

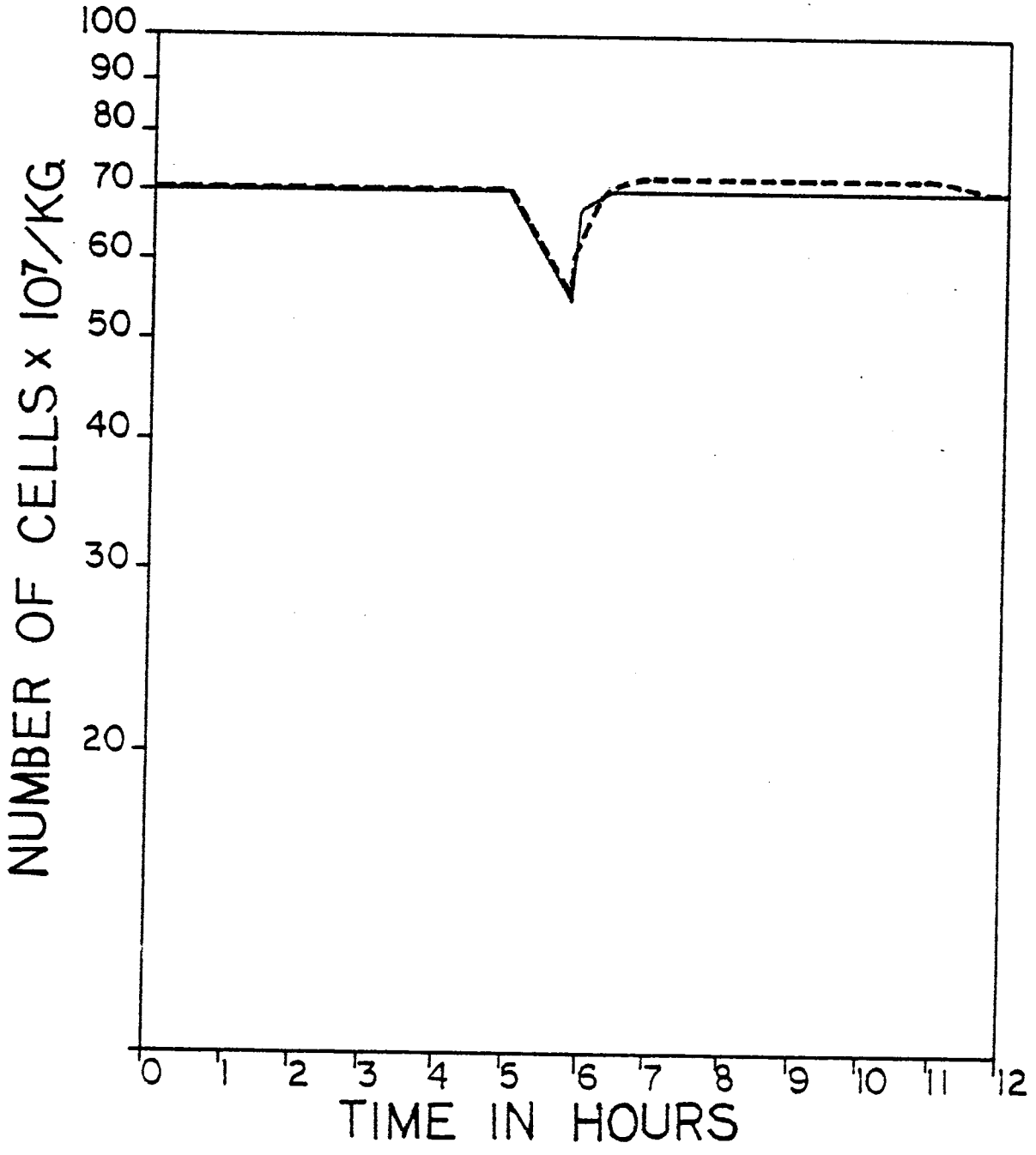


—		--	
π	0.30	π	0.30
G	0.22	G	0.12
H	0.70	H	0.70
H_2	2.45	H_2	2.45

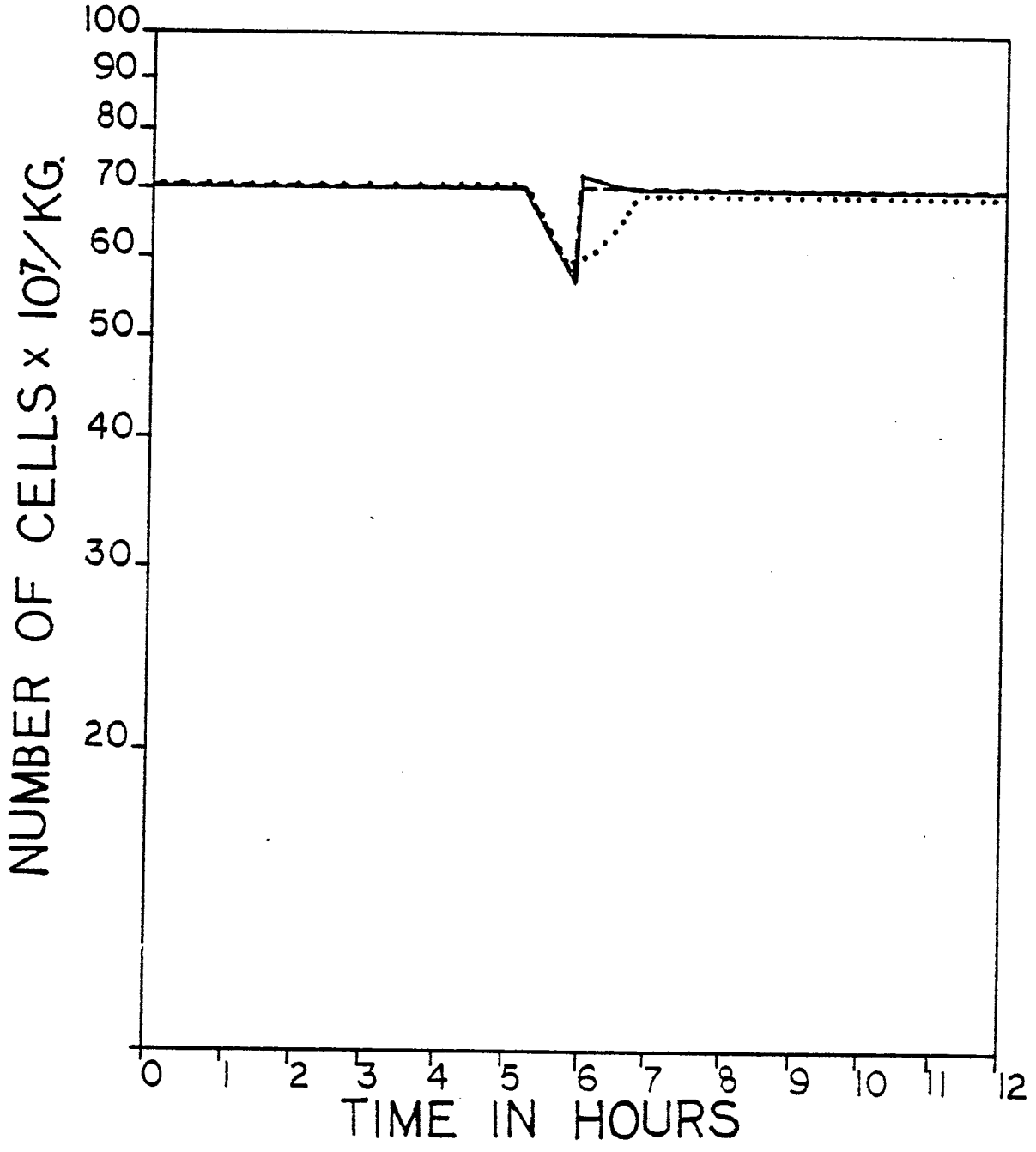

NUMBER OF CELLS x 10⁷/KG.
100
90
80
70
60
50
40
30
20
0
5
10
15
20
25
30
TIME IN HOURS



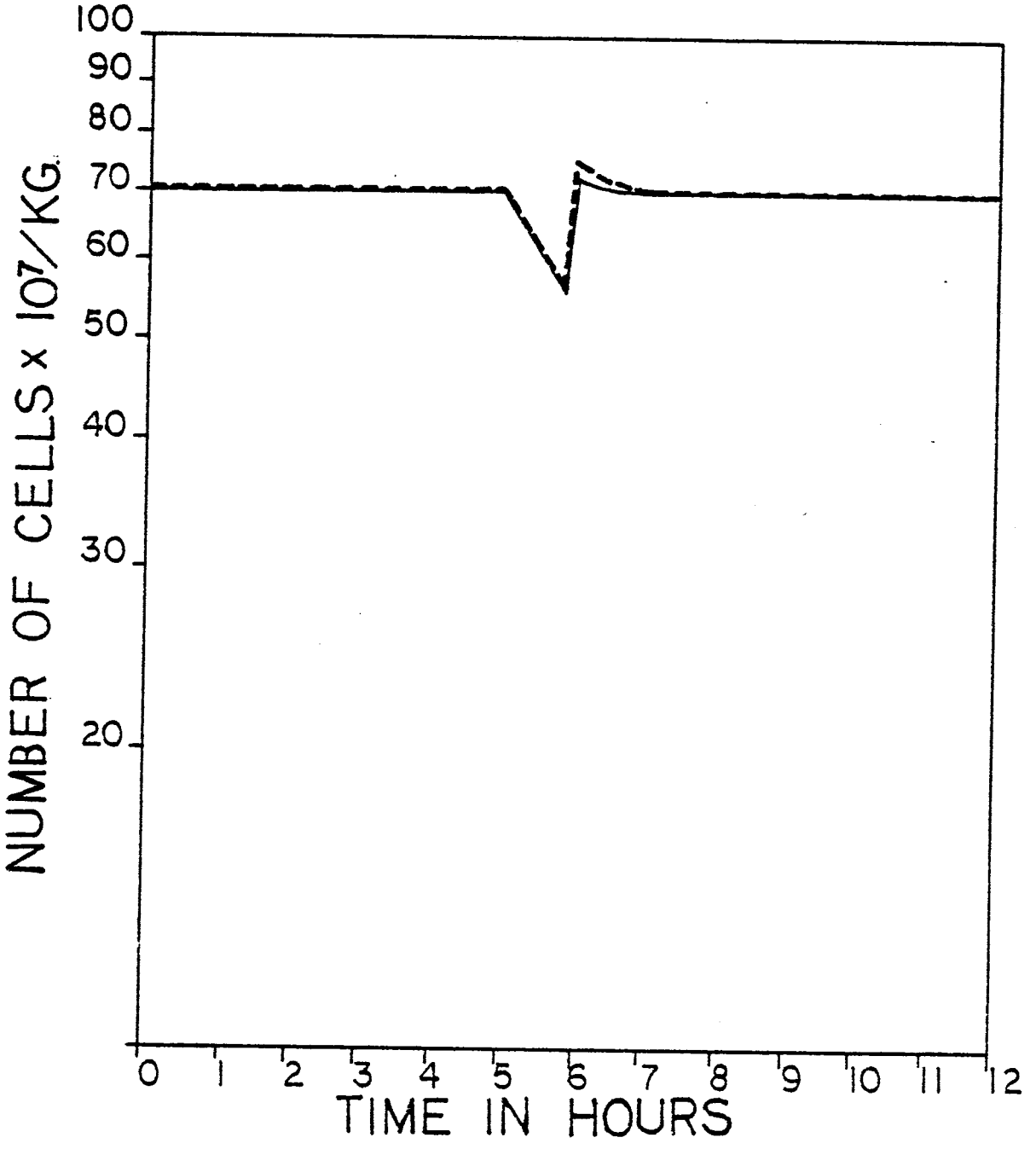
—	--	....
π 0.05	π 0.05	π 0.05
H 2.80	H 5.00	H 5.00
G 0.00	G 0.00001	G 0.001



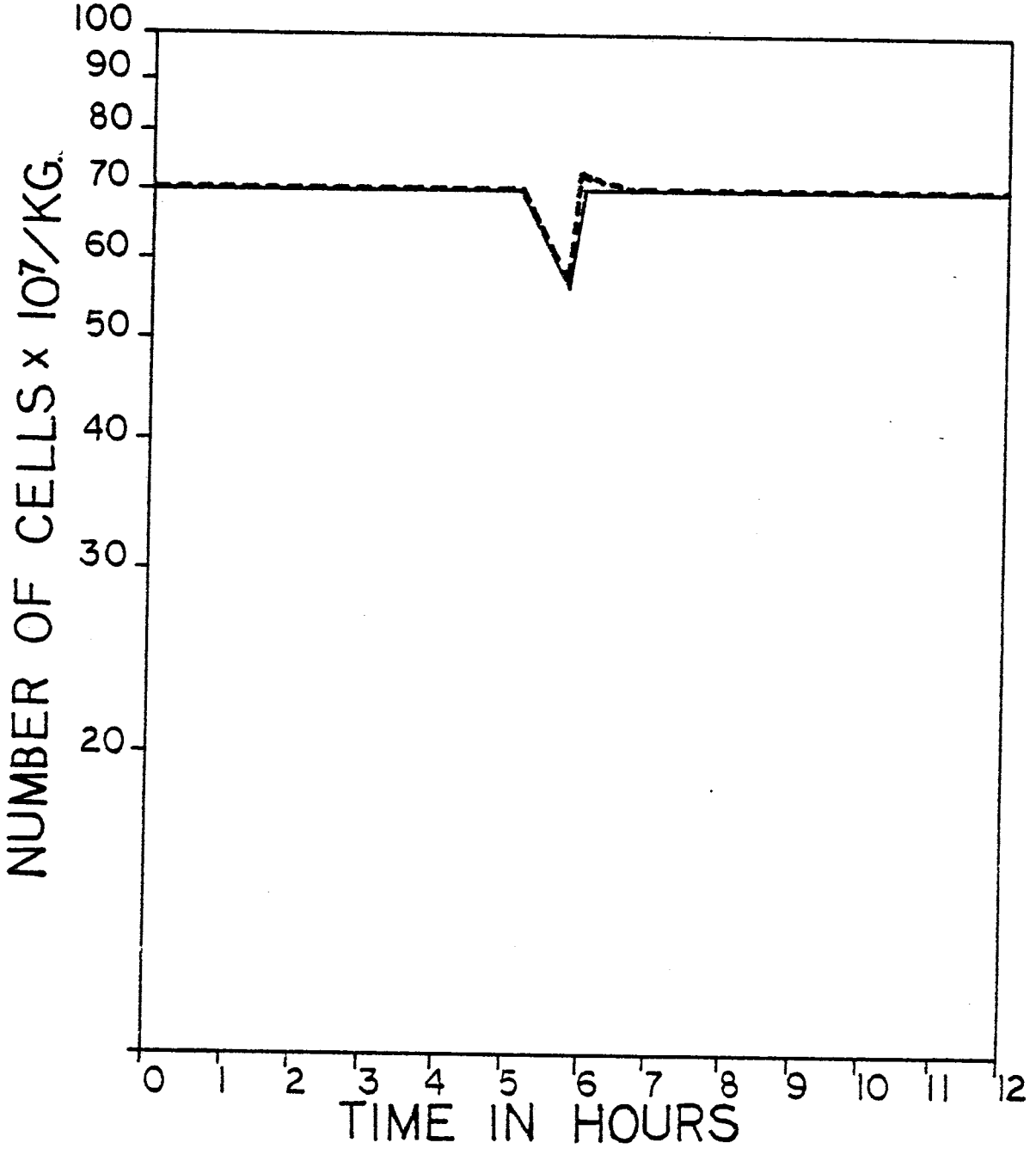
—	--
π 0.05	π 0.40
F_1 0.999	F_1 0.999
H' 5.00	H' 5.00



—	--	...
π 0.05	π 0.02	π 0.04
H 2.80	H 2.80	H 2.80
G 0.00	G 0.00	G 0.00
H_2 2.20	H_2 2.20	H_2 2.20



—	--
π 0.05	π 0.20
G 0.0068	G 0.0075
H 2.80	H 2.90
H_2 2.20	H_2 2.00



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π	0.05	π	0.40
G	0.0068	G	0.0070
H	2.70	H	2.80
H_2	2.20	H_2	2.00
G_1	0.50	G_1	0.90
H_3	0.00085	H_3	0.0030