ON THE VARIABILITY OF SURVIVAL TIMES OF MICE INOCULATED WITH CANCER CELLS

BY

ELISABETH SCHACH and SIEGFRIED SCHACH

TECHNICAL REPORT NO. 7
MAY 3, 1968

SUPPORTED BY PUBLIC HEALTH SERVICE
GRANT USPHS-GM-14554-02

DEPARTMENT OF STATISTICS
STANFORD UNIVERSITY
STANFORD, CALIFORNIA
ON THE VARIABILITY OF SURVIVAL TIMES OF MICE INOCULATED WITH CANCER CELLS

by

ELISABETH SCHACH and SIEGFRIED SCHACH

Technical Report No. 7

May 3, 1968

Prepared under U. S. Public Health Service Grant USPHS-GM-14554-02

Reproduction in Whole or in Part is Permitted for any Purpose of the United States Government

DEPARTMENT OF STATISTICS
STANFORD UNIVERSITY
STANFORD, CALIFORNIA
ON THE VARIABILITY OF SURVIVAL TIMES OF
MICE INOCULATED WITH CANCER CELLS

by

ELISABETH SCHACH and SIEGFRIED SCHACH

1. Introduction to the Problem and Description of the Data

Recently Maruyama and Brown [2] published some results on two
sets of experiments measuring the survival times of mice which had been
inoculated with tumor cells.* Two types of injections were employed.
Eighty-four mice were injected intraperitoneally with doses varying
from $10^3$ to $10^7$ cells and seventy-eight mice were injected intra-
venously with doses varying from $10^3$ to $10^6$ cells. Two animals in
the lowest dose group for intraperitoneal injection survived. Table I
and figures 1(a) and 1(b) show the mean survival times and the variances
for different doses for the two types of inoculations.

From table I and figures 1(a) and 1(b), two observations can be
made: first, for both types of inoculations the average length of time
to death decreases linearly as a function of log dose. Secondly, the
variances of the death times decrease considerably as the number of cells
in the inoculum increases. Similar results have been reported, for ex-
ample, by Meynell and Meynell [3] and Youmans and Youmans [7].

---

* More explicitly: C57 Bl/Ka female mice had been injected with
LSA ascites lymphoma. The description of the experiment may be found
on page 62 of Maruyama and Brown [2].
Figure 1: Mean survival time as a function of the log of inoculum size. Regression lines fitted by weighted least squares method.
<table>
<thead>
<tr>
<th>Initial dose</th>
<th>Intraperitoneal</th>
<th>Intravenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Mean</td>
<td>Variance</td>
</tr>
<tr>
<td>$10^3$</td>
<td>25</td>
<td>28.92</td>
</tr>
<tr>
<td>$10^4$</td>
<td>17</td>
<td>24.47</td>
</tr>
<tr>
<td>$10^5$</td>
<td>12</td>
<td>17.08</td>
</tr>
<tr>
<td>$10^6$</td>
<td>12</td>
<td>14.33</td>
</tr>
<tr>
<td>$10^7$</td>
<td>16</td>
<td>10.00</td>
</tr>
</tbody>
</table>

Since tumor cells multiply by binary fission, the exponential growth model can be expected to be a suitable mathematical model to describe the growth of the cell populations in the animals. Then, if $N_t$ is the number of tumor cells in the animal at time $t$ (t days after inoculation), we have the relationship

$$N_t = N_0 e^{ct},$$

where $N_0$ is the number of cells in the inoculum and $c$ is a constant. The generation time $T$, i.e., the time necessary for a duplication of the cell population, is then determined by

$$e^{cT} = 2$$

or

$$T = \frac{\ln 2}{c}.$$
Maruyama and Brown use this deterministic model and they make the assumption that death of the animal occurs when a fixed number $N^*$ of cells have accumulated in the host. Hence the time to death $d$ is determined by

$$N_0 e^{cd} = N^*$$

or

$$d = \frac{\ln N^* - \ln N_0}{c}.$$

On the basis of these assumptions we obtain a linear relationship between the log of the inoculum size and the survival time. Figures 1(a) and 1(b) show that for the average death times such a linear relationship holds.

The exponential growth model is a deterministic model. In its pure form it does not allow for any variation of the death times. However, table I shows that for low inoculum sizes there is considerable variation around the mean and that the variances decrease rapidly with increasing dosage. Several sources of variability come to one's mind as a possible explanation for the observed variation. In this paper we formulate several stochastic variations of the exponential growth model and we study to what extent they "explain" the variation observed in the data.

2. **Sources of Variation of Death Times**

In this section we analyze, one at a time, the effects of five
major sources of variability on the magnitude and the pattern of the
variances of the survival times.

(i) Variable Lethal Cell Number

Assume that $N^*_i$, instead of being a constant, is a random
variable which takes on the value $N^*_{ij}$ for the $j^{th}$ mouse
in the $i^{th}$ inoculum size group.\footnote{We will use two indices to identify a particular experimental
animal: the first index (i) stands for the inoculum size group,
i.e., animals in group $i$ are inoculated with $10^{2+i}$ tumor cells
($i = 1 \leq i \leq 5$); the second index ($j$) indicates a specific animal
in that group.} We assume further that
the distribution of $N^*$ is the same for all animals in all
groups. Then from (5) we obtain

\begin{equation}
\text{Var}(d_{ij}) = \frac{1}{c^2} \text{Var}(\ln N^*_{ij}) = \frac{1}{c^2} \text{Var}(\ln N^*).
\end{equation}

Thus the variances of the survival times are constant, in
particular they do not depend on the inoculum size. Vari-
ability of $N^*$ can therefore not explain the observed vari-
ance pattern.

(ii) Variable Delay Times

It could be argued that growth of the tumor does not start
immediately after inoculation, because the majority of cells
in the inoculum has to settle down at a favorable site before
the growth process can begin. If we assume that there is a
random delay time, $y_{ij}$, before growth sets in, and if the
distribution of this delay time is independent of the inoculum size, then using (5) we obtain the observed survival times

\[ d_{ij} = \frac{1}{c} (\ln N^* - \ln N_0) + y_{ij}. \]

Hence

\[ \text{Var}(d_{ij}) = \text{Var}(y_{ij}) = \text{Var}(y) \]

which is again independent of the inoculum size. Thus variable lag times for tumors produce a variance pattern which is not consistent with the data.

(iii) Poisson Distributed Inoculum Size

Tumor cell suspensions for inoculation are obtained by a dilution process. It can therefore not be assumed that the actual number of cells in the inoculum is equal to the expected number. A well-known result in probability theory states that under such conditions the actual size of the inoculum has a Poisson distribution with a parameter equal to the expected cell number (Feller [1], pages 149 and 153-4). Since the coefficient of variation of a Poisson distribution with parameter \( \mu \) is equal to

\[ \text{c. v.} = \frac{\sqrt{\mu}}{\mu} = \frac{1}{\sqrt{\mu}}, \]
it follows that the relative variation decreases as the expected cell number in the inoculum increases. However, an elementary calculation shows that Poisson variation of the inoculum size can not, under any reasonable circumstances, explain the observed variances of the survival times. Let us consider, for example, the intraperitoneal injection with the lowest concentration (expected number of cells = 10^3). From table II we get 1.33 days as an estimate for the generation time. The response group under consideration consists of 25 animals. The two extreme survival times are 17 days and 55 days. If we assume exponential growth and a fixed $N^*$, we have to conclude that the animal with a survival time of 55 days needed 38 days to reach a cell population as large as the inoculum size for the animal with the

<table>
<thead>
<tr>
<th>Type of inoculation</th>
<th>$\log \hat{N}^*$</th>
<th>$\hat{c}$</th>
<th>$\hat{T}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraperitoneal</td>
<td>9.25</td>
<td>.52</td>
<td>1.33</td>
</tr>
<tr>
<td>Intravenous</td>
<td>9.39</td>
<td>.98</td>
<td>.71</td>
</tr>
</tbody>
</table>

\(^1\) $\log \hat{N}^*$ is the point where the least squares regression line (figures 1(a) and 1(b)) intersects the x-axis. $c$ has been estimated from the slope of the linear relationship between death time and log dose (5). We used (3) to estimate $T$ from $\hat{c}$. 
shortest survival time. Thirty-eight days correspond to about 28.5 doubling times, and hence the ratio of the two extreme inocula sizes is $1:2^{28.5} \approx 1:400$ million. Since the smaller inoculum has to contain at least 1 cell, the range of the inocula sizes has to be at least in the order of magnitude of 400 million. The standard derivation of a Poisson distribution with parameter 1,000 is 31.6. Hence the range of our sample of 25 observations from a Poisson distribution would have to cover an interval of at least 12.5 million standard derivations. Chebyshev's inequality tells us that such a result is virtually impossible.\footnote{Another way of demonstrating that the present model is inappropriate is this: For all practical purposes (i.e., with very high probability) it can be assumed that all the 25 inocula sizes for this response group are within, say, 10 standard derivations from the mean; that is, they are in the interval [684, 1316]. But since it takes a cell population little more than 1 day to double, the difference between the largest and the smallest survival time will not be much more than 1 day, whereas the observed range is 38 days.} For other inocula sizes the results of similar computations are not always as extreme as this one, but the conclusion is still the same. These considerations also rule out the following model which is a slight generalization of the one described above: assume that the number of cells in the $i^{th}$ response group has a Poisson distribution with parameter $\mu = 10^{2+i}$, and that furthermore each cell has probability $\pi$ ($0 < \pi \leq 1$) of being capable of reproduction. According to Feller [1],
page 269 ff. under these conditions the number of viable cells has again a Poisson distribution but with parameter $\pi \mu$. Since the corresponding standard deviation $\sqrt{\pi \mu}$ is $\leq \sqrt{\mu}$, calculations similar to the ones above would yield even more extreme results. Thus we are led to conclude that Poisson variation alone "explains" only a negligibly small part of the observed variances of the survival times.

(iv) Variable Time Intervals Between Successive Divisions of Individual Cells

All the models considered so far introduced variation either at the end (variable N*) or at the beginning (variable delay times, variable number of viable cells) of the growth process of the tumor population. Apart from this random element the tumor cell population was assumed to follow an exponential path. This assumption is justified if the time intervals between successive cell divisions are the same for all cells and for all stages of the tumor. It seems to be more realistic to consider the time intervals between successive divisions to be random, therefore we now analyze a model which takes into account the stochastic nature of these intervals. The assumptions for the stochastic process we will consider can be put into this form:

(a) At any time-point the individual cells of the population act independently of each other.
(b) During a short time interval \( \Delta t \) any given cell has probability \( \lambda \Delta t \) of dividing and \( \mu \Delta t \) of dying (\( \lambda \) and \( \mu \) are some constants). With probability \( 1 - (\lambda + \mu)\Delta t \) the cell will neither die nor multiply.

A stochastic process which is governed by these rules is called a birth and death process. Its usefulness for the study of the growth of cell populations has been studied extensively by Shortley and Wilkins [6]. The process comes to an end when either the number of viable cells is zero (the cell population dies out) or when it reaches \( N^* \) (response: death of the animal). Therefore 0 and \( N^* \) are called absorbing states.

Shortley [5] computed the probability of response and, given that response occurs, the mean, variance and skewness of the survival times as a function of the inoculum size. He based his computations on results obtained by Saaty [4], but he achieved a considerable simplification by introducing the additional assumption that the actual size of the inoculum is Poisson distributed. (For inocula sizes \( \geq 10^3 \) this additional assumption does not have much influence on the results, since the relative variation of the Poisson distribution is then almost negligible).

Shortley obtains the result that under the above assumptions the average survival time is linearly related to the log of
the expected inoculum size.\textsuperscript{1} Hence in this respect our data coincide quite well with this theoretical model. However, we are primarily interested in the variances. Shortley derives formulas for the first three moments of the survival time distribution, but these formulas are quite involved and we use instead his graphical presentation which conveniently summarizes the relationship between the expected initial dose, \( \lambda, \mu \) and the standard deviation of the survival times (Shortley [5], Fig. 4, page 572). From this figure we obtain table III.

\begin{center}
\textbf{TABLE III}
\end{center}

\begin{center}
\textbf{Standard Deviations of the Survival Times for the Birth and Death Model}
\end{center}

\begin{center}

\begin{tabular}{|c|c|}
\hline
\textbf{Expected initial dose} & \textbf{\((\lambda-\mu)\times\sigma\)} \\
\hline
10* LD50 & .7 \\
100* LD50 & .2 \\
1000* LD50 & .06 \\
\hline
\end{tabular}
\end{center}

In order to compare these values with the present data we need some information about the LD50 and \( (\lambda-\mu) \). For the intravenous injection Maruyama and Brown estimated the median effective dose [2, table III, p. 67]. They obtained LD50 \( \approx \) 100 cells. Shortley and Wilkins show that \( (\lambda-\mu)^{-1} \) can be estimated by the slope of the line of figure 1(b) when the survival times are plotted against the natural log of the

\textsuperscript{1} For inoculum sizes exceeding 2*LD50.
inoculum size. From our data we get an estimate of \((\lambda-\mu)\) equal to .98 (from table II). Using this estimate we compute the entries of table IV.

**TABLE IV**

Theoretical and Observed Standard Deviations of Survival Times for Intravenous Inoculation

<table>
<thead>
<tr>
<th>Expected Initial dose</th>
<th>Theoretical Standard Deviation</th>
<th>Observed Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^3)</td>
<td>.714</td>
<td>1.237</td>
</tr>
<tr>
<td>(10^4)</td>
<td>.204</td>
<td>1.127</td>
</tr>
<tr>
<td>(10^5)</td>
<td>.061</td>
<td>.825</td>
</tr>
</tbody>
</table>

Since the theoretical standard deviations are considerably smaller than the observed standard deviations, we have to conclude that for the intravenous inoculation the birth and death model does not adequately describe the variation of the survival times.

For the intraperitoneal inoculation no estimate of LD50 seems to be available; therefore we cannot compute the corresponding entries in table IV. However, Shortley and Wilkins show that for any inoculum size \((\lambda-\mu)\sigma\) is less than 1.3. \((\lambda-\mu)\) has been estimated to be .52 for the intraperitoneal injection. Hence the standard deviation of the survival times should never exceed \(.7/.52 = 1.35\). From table I it can be seen that the observed standard deviations are considerably larger than this number for all but the very highest inoculum
size. Hence for the intraperitoneal inoculation we can draw
the same conclusion as for the intravenous inoculation.
It could be argued that the birth and death process is inad-
quate to describe the growth of a cell population, because it
is time-homogeneous; i.e., the probability of dividing or
dying of a cell during an interval \([t, t+\Delta t]\) is \(\lambda \Delta t\) and
\(\mu \Delta t\), respectively, for any given cell in the population,
irrespective of time \(t\). In reality it can be expected that
as \(t\) becomes larger, and as the tumor grows bigger, the rate
of growth will somewhat slow down. But as long as this deceler-
ation is about the same for all animals, it will not substan-
tially increase the variances.

(v) Variable Generation Time

We have now seen that even if we take into account the fact
that the times between successive divisions of a cell are of
a stochastic nature, the observed variation of the death times
is still much greater than the variation to be expected under
the model that we have studied. It can be shown \([1, p. 411]\)
that the birth and death model is similar to the deterministic
exponential model \((1)\) in the sense that the expected tumor
size \(E_{N_t}\) has the form

\[
E_{N_t} = N_0 e^{ct}, \quad c = \lambda - \mu
\]

(for \(t\) small enough so that the probability of absorption

13
at \( N^* \) can be neglected, i.e., the individual paths fluctuate around the exponential curve, but the average of all the conceptual paths coincides with the deterministic model (1) and the average growth factor \( c \) is the same for all cell populations.

If we give up the idea that the average generation time is the same for each population, that is, if we allow for a variable generation time which takes on different values for different populations (hosts), then we see immediately that minor variations of generation times will cause small or sizable variations of survival times, depending on whether the average survival time is small or large. It should be observed that generation times might vary not only because the hosts might differ in their ability to suppress tumor growth ("host heterogeneity"), but also because the tumor cells might settle in different locations of the host's body ("site heterogeneity"). This remark seems to be particularly relevant for the intraperitoneal type of inoculation. This might explain the difference in magnitude of the variances between types of inoculation (table I).

We now assume that for each mouse the tumor cell population increases strictly exponentially from the initial dose to \( N^* \), but that the generation time \( T_{ij} \) of the cell population (\( j^{th} \) animal in \( i^{th} \) group) varies from population to popula-
tion. We also assume that all the $T_{ij}$ have the same distribution with $E T_{ij} = \mu$, $\text{Var}(T_{ij}) = \sigma^2$. Then we get from (3) and (4)

\begin{equation}
N^* = 10^{i+2} \exp\left(\frac{d_{ij} \ln 2}{T_{ij}}\right)
\end{equation}

or

\begin{equation}
\ln N^* = (i+2) \ln 10 + \frac{d_{ij} \ln 2}{T_{ij}}
\end{equation}

and finally

\begin{equation}
d_{ij} = \frac{\ln N^* - (i+2) \ln 10}{\ln 2} T_{ij}.
\end{equation}

Hence

\begin{equation}
E d_{ij} = \frac{\ln N^* - (i+2) \ln 10}{\ln 2} \mu
\end{equation}

which is linear in $i$, and thus it is in accordance with the results presented in figures 1(a) and 1(b). Also

\begin{equation}
\text{Var}(d_{ij}) = \left(\frac{\ln N^* - (i+2) \ln 10}{\ln 2}\right)^2 \sigma^2,
\end{equation}

i.e., the variances follow a parabolic pattern and the parabola touches the x-axis at the point $\log N^*$.

Figures 2(a) and 2(b) show the observed variances and the parabola (15) fitted by the method of weighted least squares.

As compared to the other models the assumption of a variable generation time seems to be in reasonable accordance with the observed data.
Figure 2(a): Log of cell numbers in inoculum

Figure 2(b): Log of cell numbers in inoculum

Figure 2: Variance of survival time as a function of the log of inoculum size.
3. **Conclusion**

This paper deals with the variability of survival times of tumor-inoculated mice. It can be expected that the observed variability is the result of a combination of several sources of variation. We discussed some of these sources. To simplify matters we analyzed them one at a time. It is surprising that some of the sources of variation that come to one's mind rather easily do not explain more than a negligibly small part of the total variance. It seems to us that the conclusion that some model assumptions are not consistent with the observations, is more reliable than the result that another model "explains" the data. After all, there is always more than one "simple" model consistent with a given set of observations.

**Acknowledgement**

The authors are grateful to Professor B. W. Brown for suggesting the problem and for many helpful comments.
REFERENCES


