MAXIMUM LIKELIHOOD ESTIMATION OF SURVIVAL CURVE PARAMETERS

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SUMMARY

A maximum likelihood procedure is presented for the estimation of the parameters in a survival curve which is used in the quantitative investigation of cytological damage resulting from ionizing radiation. This estimation procedure is developed under the assumption that the observations are distributed as independent Poisson random variables. In addition, a weighted least squares procedure, which gives estimates equivalent to the maximum likelihood estimates, is presented. Tests of the model and of the assumed distribution of the observations are given. Two illustrative examples are included.

1. INTRODUCTION

In the quantitative investigation of cytological damage resulting from ionizing radiation, the survival curve has been extensively used in attempts to establish relations between the radiation dose and the response of the biological system. Statistical methods in common use today for obtaining estimates of survival curve parameters and their standard errors were first presented by Kimball [1953] who employed a least squares procedure. Gurian [1956] extended Kimball's procedure by proposing appropriate weights for the least squares estimation. The purposes of this paper are to obtain estimates of these parameters with the help of the maximum likelihood principle, to show that equivalent estimates can be obtained with a properly weighted least squares approach, and to propose a procedure for testing the adequacy of the model being used.

In determining the effect of radiation on bone marrow stem cells, bone marrow cells from irradiated animals are injected into recipient irradiated animals (Till and McCulloch [1961]). The injected cells locate in the spleen where the viable stem cells divide and produce

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clonal colonies. The recipient animal is sacrificed after sufficient time has elapsed for the colony to grow to macroscopic size. The colonies in the excised spleen are then counted. It is assumed that each surviving stem cell that reaches the spleen has produced one colony. Although this discussion is restricted to in vivo mammalian cell survival, the same method of estimation is clearly applicable to in vitro survival studies of microorganisms that use the culture plate method.

Since the stem cells constitute a small portion of the bone marrow cells, it is necessary to inject large numbers of bone marrow cells in order to produce colonies. It will be convenient to consider 10^5 cells as a unit concentration of bone marrow cells. Then, if there are μ stem cells/unit concentration of bone marrow cells, and some concentration say, m, of bone marrow cells is injected into a recipient animal, the number of colonies is assumed to be distributed as a Poisson variable with mean $= m\mu$. The parameter μ can be estimated by a dilution assay, and under appropriate conditions described by Roberts and Coote [1965] the maximum likelihood estimate of μ is given by $\hat{\mu}$ = total number of colonies/total concentration of cells injected.

A further assumption is that the fraction of stem cells surviving radiation dose X is of the form

$$f = 1 - (1 - e^{-\beta X})^{\nu}. \tag{1}$$

This model was originally obtained as a special case of a more general model derived from the 'target-hit' statistical theory of radiation damage when the number of 'hits' is one (Atwood and Norman [1949]). The same general equation has also been obtained from a kinetic model of radiation damage by Dienes [1966].

Although the target-hit theory remains in doubt as a representation of the lethal process in the cell, most mammalian cell survival curves resemble the target model and can be represented by the same parameters (Krebs [1967]). Krebs states that $D_0(\beta = 1/D_0$, where D_0 is the dose at which 37% survival occurs) describes the radiosensitivity of the cell, and that ν represents a threshold level of injury required for cell death. The parameter ν was originally supposed to represent the number of targets per cell, and has also been referred to as the 'extrapolation number' (Alper *et al.* [1960]). The biological significance of this parameter and the appropriateness of the model have also been discussed by Fowler [1964].

2. ESTIMATION

Assuming that equation (1) is the appropriate model, various methods have been used to estimate the parameters. These have been

discussed by Lellouch and Wambersie [1966], who proposed a maximum likelihood estimation procedure. Their method uses the approximation

$$1 - (1 - e^{-\beta X})^{\nu} \simeq \nu e^{-\beta X}$$

which requires that only that portion of the data be used for which X is sufficiently large. The following development does not require this assumption. Consequently, a 'goodness of fit' test over the entire dose range is possible, and the decision as to what dose is sufficiently large is not required.

For a given radiation dose X_i, $(i = 1, \cdots, N)$ the mean number of surviving stem cells per 10^5 bone marrow cells is given by

$$P_i = \mu[1 - (1 - e^{-\beta X_i})^{\nu}]. \tag{2}$$

If

m_{ij} = concentration of cells injected into jth animal,

n_i = number of animals at dose X_i,

y_{ij} = number of colonies observed in jth animal at dose X_i,

then it follows from the assumption of a Poisson distribution of colonies that the likelihood function is

$$L = \prod_{i=1}^{N} \prod_{j=1}^{n_i} \frac{e^{-m_{ij}P_i}(m_{ij}P_i)^{y_{ij}}}{y_{ij}!}. \tag{3}$$

The log of the likelihood function is given by

$$\ln L = -\sum_i \sum_j m_{ij}P_i + \sum_i \sum_j y_{ij} \ln (m_{ij}P_i) - \sum_i \sum_j \ln (y_{ij}!) \tag{4}$$

which may be written as

$$\ln L = -\sum_i M_i P_i + \sum_i Y_i \ln P_i + C, \tag{5}$$

where $M_i = \sum_j m_{ij}$, $Y_i = \sum_j y_{ij}$, and C is independent of the parameters.

Let $\theta_1 = \mu$, $\theta_2 = \beta$, $\theta_3 = \nu$, and $\boldsymbol{\theta}$ equal the vector of parameters, then the vector of first partial derivatives of $\ln L$ with respect to the elements of $\boldsymbol{\theta}$ is given by

$$\left[\frac{\partial \ln L}{\partial \theta_l}\right] = \left[\sum_i \frac{\partial P_i}{\partial \theta_l}\left\{\frac{Y_i}{P_i} - M_i\right\}\right] \tag{6}$$

for $l = 1, 2, 3$, and the matrix of second partial derivatives is given by

$$\left[\frac{\partial^2 \ln L}{\partial \theta_l \, \partial \theta_{l'}}\right] = \left[\sum_i \left\{\left(\frac{Y_i}{P_i} - M_i\right) \frac{\partial^2 P_i}{\partial \theta_l \, \partial \theta_{l'}} - \frac{Y_i}{P_i^2} \frac{\partial P_i}{\partial \theta_l} \frac{\partial P_i}{\partial \theta_{l'}}\right\}\right] \tag{7}$$

for $l, l' = 1, 2, 3$. Let $A = (1 - e^{-\beta X})$; then $P = \mu(1 - A^{\nu})$ and the partial derivatives in equations (6) and (7) are as follows:

$$\frac{\partial P}{\partial \theta_1} = \frac{\partial P}{\partial \mu} = 1 - A^{\nu}, \qquad \frac{\partial^2 P}{\partial \theta_1^2} = \frac{\partial^2 P}{\partial \mu^2} = 0,$$

$$\frac{\partial P}{\partial \theta_2} = \frac{\partial P}{\partial \beta} = -\mu\nu A^{\nu-1} X e^{-\beta X}, \qquad \frac{\partial^2 P}{\partial \theta_2^2} = \frac{\partial^2 P}{\partial \beta^2} = \mu\nu X^2 e^{-\beta X} A^{\nu-2}(1 - \nu e^{-\beta X}), \tag{8}$$

$$\frac{\partial P}{\partial \theta_3} = \frac{\partial P}{\partial \nu} = -\mu A^{\nu} \ln A, \qquad \frac{\partial^2 P}{\partial \theta_3^2} = \frac{\partial^2 P}{\partial \nu^2} = -\mu A^{\nu}(\ln A)^2,$$

$$\frac{\partial^2 P}{\partial \theta_1\, \partial \theta_2} = \frac{\partial^2 P}{\partial \mu\, \partial \beta} = -\nu A^{\nu-1} X e^{-\beta X} \qquad \frac{\partial^2 P}{\partial \theta_1\, \partial \theta_3} = \frac{\partial^2 P}{\partial \mu\, \partial \nu} = -A^{\nu} \ln A,$$

$$\frac{\partial^2 P}{\partial \theta_2\, \partial \theta_3} = \frac{\partial^2 P}{\partial \beta\, \partial \nu} = -\mu X e^{-\beta X} A^{\nu-1}(1 + \nu \ln A),$$

where the subscript i has been eliminated for notational convenience. In what follows it will be shown that the maximum likelihood estimates can be obtained by an iterative procedure using graphical estimates of β and ν as starting values and the control group ($X = 0$) as initial estimates of μ.

Let $\boldsymbol{\theta}^0$ be the vector of initial estimates of the parameters, $\boldsymbol{\theta}^k$ the vector of estimates on the kth iteration, and

$$\Delta\boldsymbol{\theta}^k = \boldsymbol{\theta}^{k+1} - \boldsymbol{\theta}^k. \tag{9}$$

Then the equations to be solved for $\Delta\boldsymbol{\theta}^0$ are:

$$\left[\frac{\partial \ln L(\boldsymbol{\theta}^0)}{\partial \theta_l}\right] + \left[\frac{\partial^2 \ln L(\boldsymbol{\theta}^0)}{\partial \theta_l\, \partial \theta_{l'}}\right] \Delta\boldsymbol{\theta}^0 = \mathbf{0}, \qquad l, l' = 1, 2, 3. \tag{10}$$

In place of this system we shall solve the equations

$$\left[\frac{\partial \ln L(\boldsymbol{\theta}^0)}{\partial \theta_l}\right] + \left[E\left(\frac{\partial^2 \ln L(\boldsymbol{\theta}^0)}{\partial \theta_l\, \partial \theta_{l'}}\right)\right] \Delta\boldsymbol{\theta}^0 = \mathbf{0}, \tag{11}$$

where from (7)

$$\left[E\left(\frac{\partial^2 \ln L}{\partial \theta_l\, \partial \theta_{l'}}\right)\right] = \left[-\sum \frac{M_i}{P_i}\left(\frac{\partial P_i}{\partial \theta_l}\right)\left(\frac{\partial P}{\partial \theta_{l'}}\right)\right]. \tag{12}$$

This substitution of expected for empirical values in the second-order derivatives of $\ln L$ (sometimes called the 'method of scoring') has been discussed by e.g. Finney ([1952] appendix II) and Rao ([1965] pp. 302–5).

The system of equations (12) is now solved for $\Delta\boldsymbol{\theta}^0$ and then the new vector $\boldsymbol{\theta}^1 = \boldsymbol{\theta}^0 + \Delta\boldsymbol{\theta}^0$ is substituted for $\boldsymbol{\theta}^0$ in (12). This iterative procedure is continued until the elements of $\Delta\boldsymbol{\theta}^k$ become sufficiently small.

It is possible to show that equivalent estimators may be found by a weighted least squares approach. Consider the following weighted sum of squares to be minimized:

$$\phi = \sum_{i=1}^{N} W_i(\boldsymbol{\theta}^0)(\bar{P}_i - P_i)^2, \tag{13}$$

where $\bar{P}_i = Y_i/M_i$, $W_i(\boldsymbol{\theta}^0) = M_i/P_i(\boldsymbol{\theta}^0)$, and $P_i(\boldsymbol{\theta}^0)$ is equal to P_i evaluated at $\boldsymbol{\theta}^0$. Since P_i is a nonlinear function of the parameters, it is expanded about $\boldsymbol{\theta}^0$ in a Taylor series through the linear terms and approximated by

$$P_i(\boldsymbol{\theta}^0) + \left[\frac{\partial P_i(\boldsymbol{\theta}^0)}{\partial \theta_l}\right]' \Delta\boldsymbol{\theta}^0. \tag{14}$$

This is substituted into (13) and the partials of the resulting expression with respect to the elements of $\boldsymbol{\theta}$ are set equal to zero. The resulting set of equations can be shown to be equivalent to those given in (12). Again the system of equations given in (12) is solved for $\Delta\boldsymbol{\theta}^0$ and the new vector $\boldsymbol{\theta}^1 = \boldsymbol{\theta}^0 + \Delta\boldsymbol{\theta}^0$ is substituted for $\boldsymbol{\theta}^0$ in $W_i(\boldsymbol{\theta}^0)$ and $P_i(\boldsymbol{\theta}^0)$. This iterative procedure is continued until the elements of $\Delta\boldsymbol{\theta}^k$ become sufficiently small. Hence it has been demonstrated that the weighted least squares procedure described above is equivalent to the solution of the likelihood equations discussed earlier.

The asymptotic variance covariance matrix of the estimators found by the procedures described above is given by the inverse of the matrix

$$\left[-E\left(\frac{\partial^2 \ln L}{\partial \theta_l \, \partial \theta_{l'}}\right)\right] = \begin{bmatrix} a & b & c \\ b & d & e \\ c & e & f \end{bmatrix},$$

where

$$\begin{aligned} a &= \sum \frac{M_i}{P_i}\left(\frac{\partial P_i}{\partial \mu}\right)^2, & d &= \sum \frac{M_i}{P_i}\left(\frac{\partial P_i}{\partial \beta}\right)^2, \\ b &= \sum \frac{M_i}{P_i}\left(\frac{\partial P_i}{\partial \mu}\right)\left(\frac{\partial P_i}{\partial \beta}\right), & e &= \sum \frac{M_i}{P_i}\left(\frac{\partial P_i}{\partial \beta}\right)\left(\frac{\partial P_i}{\partial \nu}\right), \\ c &= \sum \frac{M_i}{P_i}\left(\frac{\partial P_i}{\partial \mu}\right)\left(\frac{\partial P_i}{\partial \nu}\right), & f &= \sum \frac{M_i}{P_i}\left(\frac{\partial P_i}{\partial \nu}\right)^2. \end{aligned} \tag{15}$$

In practice, μ, β, and ν are replaced in the above expressions by their estimates in order to obtain estimates of the elements of this matrix.

For the case when m_{ij} is independent of j for all i, which is denoted by using m_i instead of m_{ij} , it is possible to test the assumptions of Poisson variation and the form of the model as expressed by equation (2). Let

$$\hat{P}_i = \hat{\mu}[1 - (1 - e^{-\hat{\beta}X_i})^{\hat{\nu}}]$$

be the estimated mean number of surviving stem cells per unit concentration of bone marrow cells at dose X_i . Then the statistic

$$\sum_{i=1}^{N} \sum_{j=1}^{n_i} (y_{ij} - m_i\hat{P}_i)^2/m_i\hat{P}_i \tag{16}$$

is distributed approximately as χ^2 with $\sum_{i=1}^{N} n_i - 3$ D.F. This statistic would allow for a test of the goodness of fit of the model under the assumption of Poisson variation. The expression in equation (16) may be partitioned into two independent components as shown by

$$\begin{aligned} \sum_{i=1}^{N} \sum_{j=1}^{n_i} \frac{(y_{ij} - m_i\hat{P}_i)^2}{m_i\hat{P}_i} &= \sum_{i=1}^{N} \sum_{j=1}^{n_i} \frac{(y_{ij} - \bar{y}_{i.})^2}{m_i\hat{P}_i} + \sum_{i=1}^{N} \frac{n_i(\bar{y}_{i.} - m_i\hat{P}_i)^2}{m_i\hat{P}_i} \\ &= \quad T_1 \quad + \quad T_2, \end{aligned} \tag{17}$$

where $\bar{y}_{i.} = \sum_{j=1}^{n_i} y_{ij}/n_i$. T_1 is distributed approximately as χ^2 with $f_1 = \sum_{i=1}^{N} n_i - N$ D.F. and may be used to test the assumption of Poisson variation. If this statistic is not significant then T_2 is distributed approximately as χ^2 with $f_2 = N - 3$ D.F. and may be used to test the model as given by equation (2). If the magnitude of T_1 indicates some variation additional to the Poisson variation, then, following a testing procedure similar to that presented by Roberts and Coote [1965], the appropriateness of the model may be tested by the ratio $(T_2/f_2)/(T_1/f_1)$ which is distributed approximately as a F-statistic with (f_2 , f_1) D.F. when the model is correct. If the F-statistic is not significant, then the variance-covariance matrix is multiplied by the heterogeneity factor h.f. $= T_1/f_1$ in order to obtain a more appropriate estimate.

3. EXAMPLES

In this section the estimation procedure is illustrated with two examples. In the first example some data obtained from Dr. F. Comas of Oak Ridge Associated Universities is used. In this experiment, the bone-marrow donors and the recipients were Fischer-334 rats. The

donors were irradiated with doses ranging from 100 to 500 rads and there was also a non-irradiated control group. The bone-marrow was removed from the femora and cell suspensions of the desired concentration obtained. The bone-marrow cells were then injected into the irradiated recipient animals, and on the 12th post-injection day, the recipients were killed, and the number of spleen colonies in each was counted. The results of the experiment are summarized in Table 1.

Initial estimates μ_0 , β_0 , ν_0 of the survival curve parameters are obtained by plotting the doses (X_i) against the observed spleen colonies per concentration on semilogarithmic paper (see Figure 1). From Figure 1, point B, which is observed from the control, provides the initial estimate of μ. From the slope of the hand-drawn line through the last seven points on this graph an initial estimate of β is obtained. Finally the initial estimate of ν is found by the ratio of point A to point B. The maximum likelihood estimates are then obtained by the iterative procedure discussed earlier and are as follows:

$$\hat{\mu} = 5.38 \quad \hat{\beta} = 0.00997 \quad \hat{\nu} = 3.28.$$

Figure 2 shows the original data with the fitted curve. The last column of Table 1 gives the predicted values of P_i using the calculated estimates of μ, β, and ν. The estimated variance-covariance matrix is

$$\mathbf{V} = \begin{bmatrix} 0.508 & -0.145 \times 10^{-3} & -0.522 \\ & 0.161 \times 10^{-6} & 0.279 \times 10^{-3} \\ & & 0.696 \end{bmatrix}.$$

The values of the χ^2's are given in Table 2. The first χ^2, T_1 , is significant at the 0.02 level. This indicates that there is some additional variation within dose groups additional to Poisson variation. Heterogeneity may occur as a result of differences in the animals, or errors in the experimental technique. The second χ^2, T_2 , is clearly not significant, indicating that the data fit the multi-target model well. Hence the elements of the estimated variance-covariance matrix are multiplied by the heterogeneity factor, h.f. $= T_1/f_1 = 68.07/45 = 1.513$. The new estimated variance-covariance matrix is then

$$\begin{bmatrix} 0.769 & -0.219 \times 10^{-3} & -0.790 \\ & 0.244 \times 10^{-6} & 0.422 \times 10^{-3} \\ & & 1.053 \end{bmatrix}.$$

By use of the asymptotic normality of maximum likelihood estimators approximate confidence intervals for the survival curve parameters may be calculated.

TABLE 1

SUMMARY OF RESULTS OF FIRST EXAMPLE

Radiation dose (rads)	Conc.* of cells injected into recipients	Number of colonies counted in spleen of the recipient animal	Total number of colonies	Total conc. injected	Spleen colonies per conc.	Predicted spleen colonies per conc.
X_i	m_{ij}	y_{ij}	Y_i	M_i	$\bar{P}_i = Y_i/M_i$	$\hat{P}_i$
0	4	22, 22	44	8	5.50	5.38
100	4	17, 14, 8, 16	55	16	3.44	4.19
150	6	21, 33, 16, 22, 20, 17, 18, 15	162	48	3.38	3.04
200	10	23, 18, 28, 13, 29, 23, 28, 13	175	80	2.19	2.05
250	15	20, 18, 10, 17, 25, 22, 14	126	105	1.20	1.33
300	20	10, 19, 15, 17, 16	77	100	0.77	0.84
400	50	15, 20, 15, 16, 14	80	250	0.32	0.32
450	70	10, 24, 13, 17, 13, 9, 11	97	490	0.198	0.196
500	100	7, 11, 16, 6, 21, 16, 11, 11	99	800	0.124	0.120

* Unit concentration = 10^5 bone marrow cells

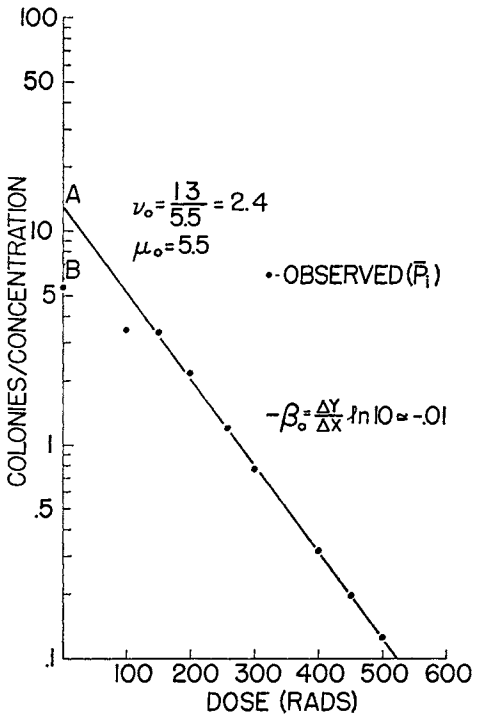

FIGURE 1

INITIAL ESTIMATES FOR THE FIRST EXAMPLE

In the second example similar data were obtained from Till and McCulloch [1961]. The maximum likelihood estimates of the parameters are $\hat{\mu} = 7.64$, $\hat{\beta} = 0.00934$, and $\hat{\nu} = 2.89$. The χ^2 values did not indicate that either the assumption of a Poisson distribution of colonies or the model should be rejected.

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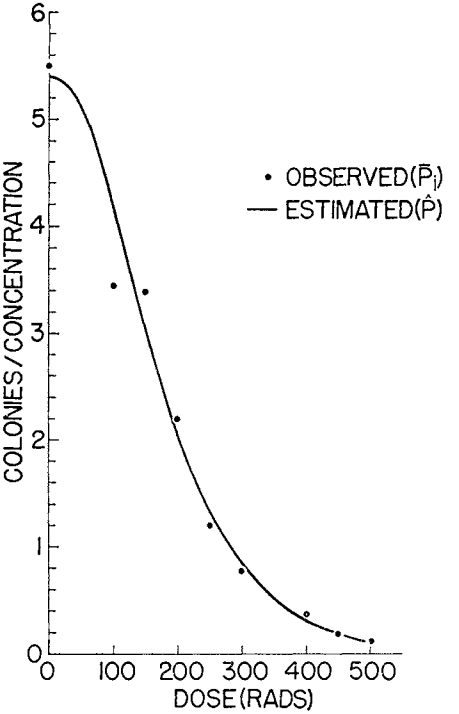

FIGURE 2

ORIGINAL DATA AND FITTED CURVE FOR THE FIRST EXAMPLE

TABLE 2

CHI-SQUARE VALUES FOR FIRST EXAMPLE

Source	D. F.	χ^2
Between	6	$T_2 = 6.612$
Within	45	$T_1 = 68.07$
Total	51	$\sum_i \sum_j (y_{ij} - m_i\hat{P}_i)^2/m_i\hat{P}_i = 74.682$

ESTIMATION DES PARAMETRES DE COURBES DE SURVIE PAR LA METHODE DU MAXIMUM DE VRAISEMBLANCE

RESUME

Une méthode du maximum de vraisemblance est présentée pour l'estimation des paramètres d'une courbe de survie qui est utilisée dans l'investigation quantitative des dommages cytologiques provoqués par des radiations ionisantes. Cette procédure d'estimation est développée sous l'hypothèse que les observations sont distribuées comme des variables aléatoires de Poisson indépendantes. De plus, une procédure des moindres carrés pondérés qui donne des estimations équivalentes aux estimations du maximum de vraisemblance est présentée. Des tests du modèle et de la distribution présumée des observations sont également donnés ainsi que deux exemples illustratifs.

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