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Nonparametric Representation of Dose–Effect Relations[1]

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KELLERER, A. M., CHMELEVSKY, D., AND HALL, E. J. Nonparametric Representation of Dose–Effect Relations. *Radiat. Res.* **84**, 173–188 (1980).

A method is described that permits a least-squares fit of survival data without invoking analytical expressions for the survival curve. As a more general constraint, applicable to a broad range of experimental data, convexity (from above) in the semilogarithmic representation is utilized. The application of the method to individual survival curves is illustrated by a numerical example. The actual purpose of the method is, however, the analysis of sets of survival curves that differ by dose-modifying factors. The need for such joint fits arises, for example, in the biological intercomparison of neutron beams for radiotherapy, or in the determination of the oxygen enhancement ratio. It is exemplified here in the application of the method to a set of repeated survival experiments for hamster cells exposed to X rays. The method provides an estimated theoretical survival curve that is based on the total set of data, and it also yields a set of dose-modifying factors that represent deviations from the ideal curve in the individual experiments.

INTRODUCTION

The representation of observed dose–effect relations by analytical expressions is often a suitable method to verify or reject models of radiation action. In certain instances, however, it introduces an undesirable bias into the experimental data, and a method of curve fitting that is more flexible than the analytical representation, but less arbitrary than a simple visual fit to the data points, is then desirable. Such a method will be described.

For simplicity the discussion will refer to survival curves of irradiated cells, but it will be evident that the considerations apply to dose–effect relations in general, or even to other relations such as the dependence of observed effects on time.

The method will first be exemplified by its application to individual survival curves. However, individual survival curves will usually not necessitate sophisticated methods of fitting the data. Application of the method to individual survival

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curves is therefore of less practical importance than the simultaneous fit of sets of survival curves that differ by dose-modifying factors.

CONDITIONS FOR CURVE FITTING

The Least-Squares Criterion

Curve fitting is the determination of a theoretical survival function $S(D)$ that is in "closest agreement" with observed data. The observed data are survival levels s_i experimentally obtained at different doses D_i ($i = 1, 2, \ldots, I$). One or more of these doses may be zero; i.e., the corresponding observations may be controls. It will be assumed that the doses are arranged in ascending order ($D_{i+1} \geq D_i$).

The notion of closest agreement can be formulated in terms of different criteria, the two most suitable being the *maximum likelihood* and the *least-squares criterion*. The least-squares criterion only will be utilized here. In the case of survival curves based on sufficient numbers of surviving colonies, the two criteria are asymptotically equivalent. The maximum likelihood criterion is considered in Appendix B.

It will be practical to refer to the logarithm of survival rather than to survival, and appropriate abbreviations will therefore be used. The logarithms of the observed survivals are designated

$$t_i = \ln s_i \qquad (i = 1, 2, \ldots, I). \tag{1}$$

The corresponding theoretical estimates of the logarithms of survival resulting from the curve-fitting procedure are designated

$$T_i = \ln S_i, \tag{2}$$

where S_i is an abbreviation for the estimated survival $S(D_i)$ at dose D_i.

For brevity t_i and T_i will be called *observed* and *theoretical survival levels*, while in fact they are the logarithms of the observed and estimated survival levels.

The symbol σ_i will stand for the estimated standard error of the logarithm of survival at dose D_i. In Appendix A the relation between the variances σ_i^2 of the logarithms and the variances of the linear survival ratios is given.

With these abbreviations, the least-squares condition is

$$\sum_{i=1}^{I} (T_i - t_i)^2/\sigma_i^2 = \phi(T_i) = \text{Min}; \tag{3}$$

i.e., one must ask for those values T_i that minimize the function $\phi(T_i)$.

Equation (3) involves the estimated survivals T_i at the dose values D_i. Only the array T_i ($i = 1, 2, \ldots, I$) of estimated survival levels will therefore be derived, and not the complete function $T(D)$. Provided the number of data points is not too small, the set of estimated survival values T_i ($i = 1, 2, \ldots, I$) will determine the other values $T(D)$ closely; even a simple polygon through the estimated points will then be an adequate representation of the whole survival curve. The least-squares condition is meaningful only if one excludes the trivial solution $T_i = t_i$. This solution is usually unacceptable since it does not lead to a "smooth" curve. Certain restrictions must therefore be applied to the possible values T_i to ensure a smooth curve.

The common method of introducing such constraints is the assumption of a specified analytical expression for $T(D)$. Examples are a linear–quadratic expression in D or the well-known, more complicated relation that derives from the so-called multitarget equation. By the choice of an analytical expression the possible sets of values T_i are restricted, and the degree of restriction depends on the type of the equation that is chosen. If the equation contains only one or two free parameters it can often provide a meaningful test of a model that corresponds to the equation. If more free parameters are involved, most observed survival data can be approximated, and a fit cannot be interpreted as confirmation of the particular model underlying the equations. Instead the fit is merely an approximation of the actual survival curve that may be utilized for comparison of data obtained under different conditions. In this case it is more desirable to avoid the use of analytical expressions altogether to eliminate the bias that is caused by the choice of such expressions.

The question then arises: How can suitable constraints for the possible sets of values T_i be formulated? Furthermore, if such constraints are found, a mathematical procedure will be required that permits the determination of those values T_i ($i = 1, 2, \ldots, I$) that minimize Eq. (3) while satisfying the constraints. These problems will be considered.

Constraints

Suitable constraints must ensure that the survival curve, without being restricted by some analytical expression, will nevertheless have essential properties that are common to most of the equations utilized to fit survival data.

A very general property of such equations is *monotonic* decrease of T_i with dose:

$$T_{i+1} - T_i \leq 0 \qquad (i = 1, 2, \ldots, I\text{-}1). \tag{4}$$

However, this condition is not sufficient, as it will not, in general, guarantee a smooth curve. Therefore it will not be considered further. Another property that is common to most survival equations is *convexity* (from above)[2] in the usual semilogarithmic plot. This is expressed by the following $I - 2$ constraints C_i:

$$C_i = (D_{i+2} - D_{i+1})T_i - (D_{i+2} - D_i)T_{i+1} + (D_{i+1} - D_i)T_{i+2} \leq 0$$
$$(i = 1, 2, \ldots, I - 2). \tag{5}$$

These constraints are a suitable basis for the numerical fitting of a majority of survival curves. It is evident that there are data that fit neither the classical dose–effect equations nor the more general constraint of convexity. Modifications of Eq. (5) that allow for a shallow tail of a dose–effect relation, as may result from an hypoxic fraction of cells, may then be required.

After the choice of a suitable set of constraints the problem of curve fitting is reduced to finding among all possible sets T_i ($i = 1, 2, \ldots, I$) the set of values that minimizes the least-squares function $\phi(T_i)$. This is a nonlinear optimization problem that can be solved with established numerical techniques.

[2] This somewhat arbitrary term is used for brevity. It signifies negative or vanishing curvature of the function ln $S(D)$; i.e., the area under the function ln $S(D)$ is convex.

FITTING OF INDIVIDUAL SURVIVAL CURVES

Computational Procedure

Equation (3), together with a set of constraints such as Eq. (5), can be solved by established numerical procedures such as the method of steepest descent (*4*). These are numerical iteration procedures that search for extrema of a function $\phi(T_i)$. The function need not be linear in the variables T_i, and only such variables T_i are chosen that meet the constraints.

General purpose computer versions of such algorithms exist (*1*, *7–9*). In their typical form these algorithms require four subroutines that have to be provided by the user. One of these computes the so-called *economical function* $\phi(T_i)$ for a given set of values T_i ($i = 1, 2, \ldots, I$). Another subroutine computes the partial derivatives $\partial\phi/\partial T_i$ of the economical function. A further subroutine computes the constraints C_i, and a last subroutine calculates the partial derivatives $\partial C_i/\partial T_k$ of the constraints. The relations that correspond to Eqs. (3) and (5) are listed in Appendix C to facilitate the actual use of the method.[3]

In addition, an initial set of estimated values T_i ($i = 1, 2, \ldots, I$) is required as a starting point for the algorithm. We encountered no convergence difficulties associated with starting points.

Numerical Example

Figure 1 illustrates the method by its application to a classical radiobiological study, namely, the survival data obtained by McCulloch and Till (*6*) for mouse marrow cells irradiated *in vivo*. The vertical bars represent observed survival ratios with their standard errors; the open circles correspond to the least-squares solution T_i ($i = 1, 2, \ldots, I$). The ordinate is normalized to T_1, i.e., to the theoretical value that belongs to the controls. This theoretical value need not coincide with the observed survival ratio t_1 for the controls. The difference is, however, frequently insignificant because most experiments utilize a considerable number of control dishes, and the statistical weight of the observation is therefore large enough to bring the theoretical estimate close to the observed control value.

The curve in Fig. 1 is drawn by hand through the set of estimated theoretical values T_i ($i = 1, 2, \ldots, I$), i.e., through the open circles. It is evident that this leaves little freedom for interpolation, so that the estimated survival curve is well defined.

The result of the nonparametric fit shows substantial curvature even at higher doses, while in the original publication the curve was drawn as a nearly straight line. Consistent with this difference the present analysis indicates an extrapolation number in excess of 3, while the original publication indicated a value of only 1.5. The present example demonstrates that the nonparametric fit can be useful even in its application to individual survival curves. The main purpose of the method is, however, the joint analysis of a group of similar survival curves; this will be treated next.

[3] The standard version of our program utilizes the computer algorithm GRGA developed by Abadie (*1*) for general purpose application. Other algorithms (*7–9*) can be used alternatively.

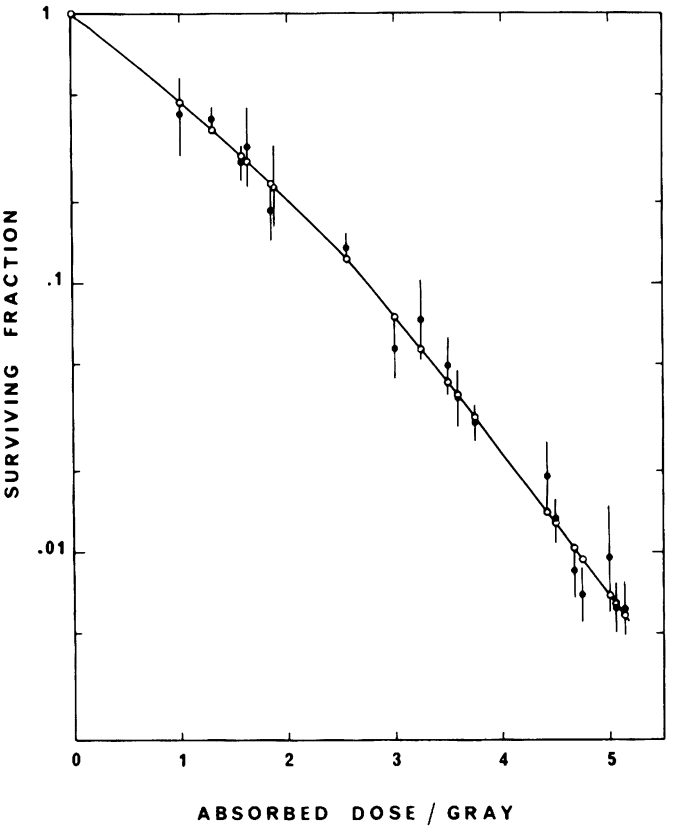

FIG. 1. Gamma-ray survival curve obtained by McCulloch and Till (*6*) for the colony-forming ability of mouse marrow cells irradiated *in vivo*. The data are given as solid circles, their standard deviations as vertical bars. The solid line represents the nonparametric fit obtained by interpolation from the theoretical values (open circles) that result from the fitting procedure. Most of the data had been obtained by irradiation after injection into the recipient mice. A few data points had been obtained by irradiation *in situ* in the femurs of living donor mice; if these latter points are disregarded, one obtains nearly the same theoretical curve.

JOINT FIT OF SEVERAL SURVIVAL CURVES

Computational Procedure

There are situations where, in one experimental investigation, several dose–effect relations are obtained that are assumed to differ only by constant dose-modifying factors. An example is the comparison of survival curves obtained with different high-energy neutron beams for therapy (*2*). The differences between such survival curves are of the order of a few percent. It may then not be feasible to resolve the dependence of the relative biological effectiveness of two such beams on dose; instead the difference in effectiveness is approximated by a constant dose-modifying factor assigned to each curve. A similar situation applies if slightly different dose–effect relations are obtained at varying positions within a phantom.

Another example is the determination of the oxygen enhancement ratio (OER). It is probable that the OER depends on absorbed dose, but in most investigations it will be impossible to resolve any dose dependence of OER. It may then be a satisfactory approximation to treat the OER as a constant dose-modifying factor.

The simultaneous nonparametric fit of several dose–effect relations that differ by dose-modifying factors will be considered in the following. The principles are the same as in the fitting of individual survival curves, but the technical details of the calculation are different and somewhat more complicated.

As in the previous section, logarithmic survival levels are considered. The index i ($i = 1, 2, \ldots, I_k$) refers again to the different doses applied in one set of irradiations that establishes one survival curve. The index k ($k = 1, \ldots, K$) stands for the number of the survival curve. For brevity k is termed the number of the experiment; i.e., the totality of data obtained in the experimental investigation is said to consist of K experiments, each of these experiments establishing one dose–effect relation. With this convention t_{ik} stands for the data point i in experiment k. Accordingly, t_{ik} is the logarithm of the number of viable colonies per plated cell at dose D_{ik}. The corresponding standard deviation is σ_{ik}.

The dose-modifying factors will be designated by f_k, and $f_k D_{ik}$ will be called the effective dose. Since the f_k are relative factors, one may first set $f_1 = 1$ and subsequently renormalize the values f_k relative to their average. The objective of the fitting procedure consists of finding dose-modifying factors and the dose–effect relation $T(D)$ that minimize the sum of the squared deviations:

$$\sum_{ik} (T(f_k \cdot D_{ik}) - t_{ik})^2/\sigma_{ik}^2 = \phi = \text{Min.} \tag{6}$$

In analogy to the procedure described in the preceding section one could derive, in addition to the factors f_k, all theoretical values $T(f_k \cdot D_{ik})$. However, this is impractical if a considerable number K of experiments are to be fitted simultaneously. A modified method is therefore more convenient, where theoretical survival levels are estimated for a preselected, equidistant grid of values of the effective dose, and where the squares of the deviations are calculated on the basis of values $T(f_k \cdot D_{ik})$ obtained by linear interpolation between the grid points.

In the present context it is sufficient to outline the essentials of the mathematical procedure. For the actual numerical execution one can utilize the formulas listed in Appendix D.

The variables which will be optimized are the theoretical survival levels on an equidistant grid $n \cdot \Delta$ ($n = 0, 1, 2, \ldots, N$) of effective doses that are multiples of a convenient increment in dose, such as $\Delta = 1$ or 0.5 Gy.

The variables obtained in the optimization procedure are the values T_n ($n = 1, \ldots, N$) at the grid points and the dose-modifying factors f_k ($k = 2, \ldots, K$).

This is the situation where the same survival level (plating efficiency) results for unirradiated controls ($D = 0$) in the different experiments. In certain experiments (OER determinations) the conditions for the controls are not the same. In these cases one will have to include survival-modifying factors, i.e., vertical shifts for the individual survival curves. These are then additional variables in the optimization procedure; they are considered in the formulas in Appendix D. In practice the

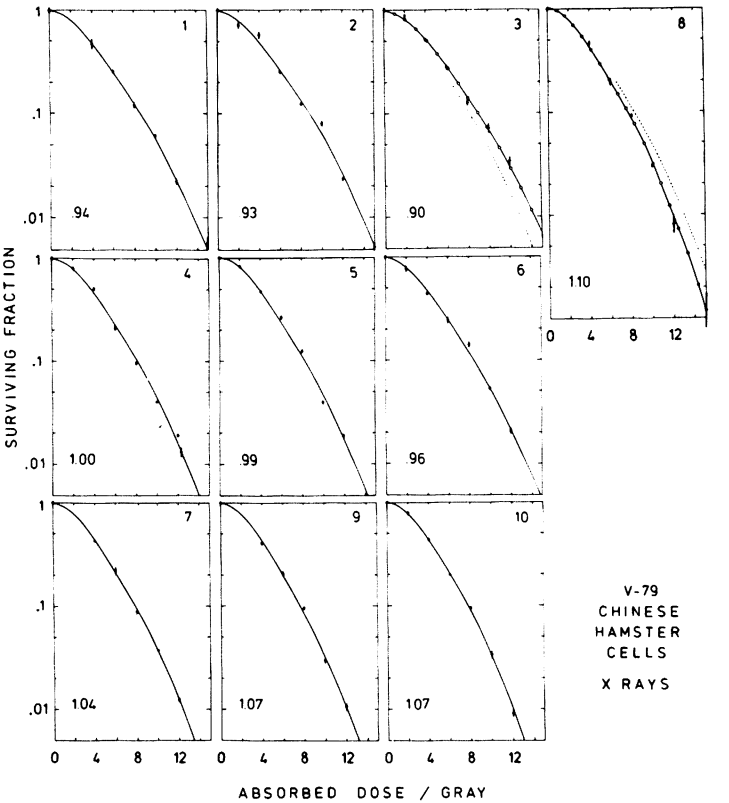

FIG. 2. A series of independently established X-ray survival curves for V-79 hamster cells (*3*). The data are given as solid dots, their standard deviations as vertical bars. The joint nonparametric fit results in the solid curves. The dose-modifying factors which are a measure for the relative sensitivity of the cells are given on the lower left corner of the panels. The number of the experiment is given in the upper right corner. The experiments have been performed over a period of several months concurrently with neutron experiments. The numbering corresponds to the order of the curves given in Table III of the earlier analysis (*5*). The estimated theoretical survivals on the equidistant grid of doses are indicated by a series of open circles for experiments 3 and 8. Furthermore, the average survival curve (dose-modifying factor 1) is added as a broken line for these two experiments.

vertical shift factors are usually of minor importance as they are close to unity after each curve is normalized to its own control.

Numerical Example

The nonparametric curve-fitting procedure will be exemplified here by its application to 10 sets of repeated X-ray survival data for V-79 hamster cells. These data were obtained by Hall *et al.* (*3*) as concurrent control observations in an investigation of the effectiveness of neutrons of different energies. The experiments with various neutron energies had to be spread over a period of several months; accordingly, it was judged desirable to determine simultaneously with each neutron experiment X-ray survival data that could indicate possible fluctuations in

TABLE I

Dose-Modifying Factors and Their 95% Confidence Ranges for 10 X-Ray Survival Curves (see Fig. 2) and Comparison with the Results from an Earlier Analysis (5)

Number of experiment (k)	*Dose-modifying factors*		
	f_k	*95% Confidence range*	f_k *From earlier analysis (5)*
1	0.944	0.930–0.957	0.95
2	0.932	0.908–0.958	0.94
3	0.898	0.870–0.925	0.90
4	1.002	0.979–1.020	1.01
5	0.995	0.963–1.032	0.99
6	0.961	0.938–0.982	0.96
7	1.044	1.023–1.061	1.06
8	1.095	1.078–1.111	1.065
9	1.067	1.038–1.095	1.075
10	1.067	1.049–1.091	1.05

the sensitivity of the cells. Such fluctuations from experiment to experiment have indeed been found, and in an earlier analysis of these data (5) it was concluded that they correspond to dose-modifying factors in the approximate range of 0.94 to 1.06. The earlier analysis was based on the determination of the mean inactivation dose for each survival curve, i.e., on one numerical parameter for each curve. It is therefore of interest to examine whether a joint nonparametric fit of the set of curves leads to results consistent with those of the earlier analysis, and whether one idealized survival curve together with the set of modifying factors can adequately represent the data.

Figure 2 gives the results of the joint fit of all 10 X-ray survival curves. The vertical bars represent the observed data and their standard deviations. The solid curves result from the ideal survival curve by dividing the abscissa values by the dose-modifying factor. Although this is somewhat arbitrary, the curves are plotted so that the observed controls lie on the origin; accordingly, the theoretical curves need not pass exactly through this point. However, the vertical shifts are so small that in most cases the theoretical curves pass virtually through the control values.

As pointed out previously, the estimated curves are obtained by an interpolation of the estimated survivals on an equidistant grid of doses. For experiments 3 and 8 this grid of estimated survivals is indicated as a series of open dots for a grid distance $\Delta = 1$ Gy. Computations with a finer grid of $\Delta = 0.5$ Gy lead to no appreciable changes in the numerical results. It will be noted that the estimated curve is closely determined even by grid points with $\Delta = 1$ Gy. A degree of uncertainty remains in the initial slope of the curves, but any precise value of the initial slope would be a mathematical artifact in this experiment that includes no doses below 2 Gy.

The dose-modifying factors that result from the analysis are a measure of the relative sensitivity of the cells; these factors are listed on the bottom left corner

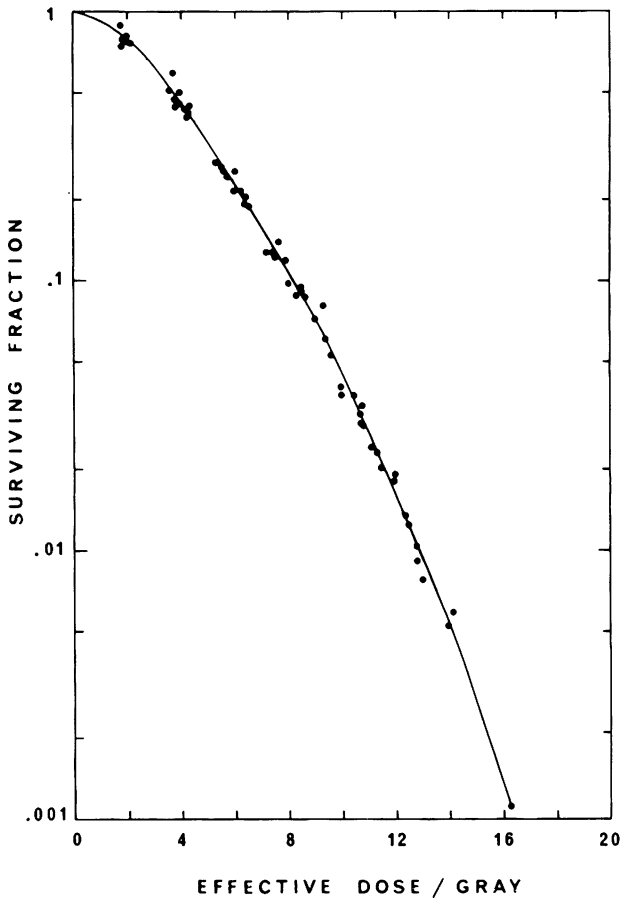

FIG. 3. The data from Fig. 2 rescaled according to the dose-modifying factors. The solid curve is the theoretical survival curve that results from the nonparametric fit; it corresponds to the broken lines in Fig. 2.

of each panel. The factors differ appreciably. As indicated in Table I they are consistent with the values obtained in the earlier analysis (*5*).

The derivation of confidence limits for the estimated survival curves is problematical, because it is not clear how many degrees of freedom can be assigned to the fitting procedure if, in fact, the notion of degrees of freedom is applicable at all. This will require further study. However, confidence limits are given in Table I for the dose-modifying factors, i.e., for the actual values of interest. Their derivation is explained in Appendix E.

The approximation of all observations by an ideal curve adjusted by dose-modifying factors is, judged from Fig. 2, consistent with the data. A somewhat more direct indication of the overall fit is afforded by Fig. 3, which gives the estimated ideal curve together with all data in a plot where the *data* are adjusted by the modifying factors; in this plot the abscissa is not the absorbed dose but the *effective* dose, i.e., the absorbed dose multiplied by the dose-modifying factor. For

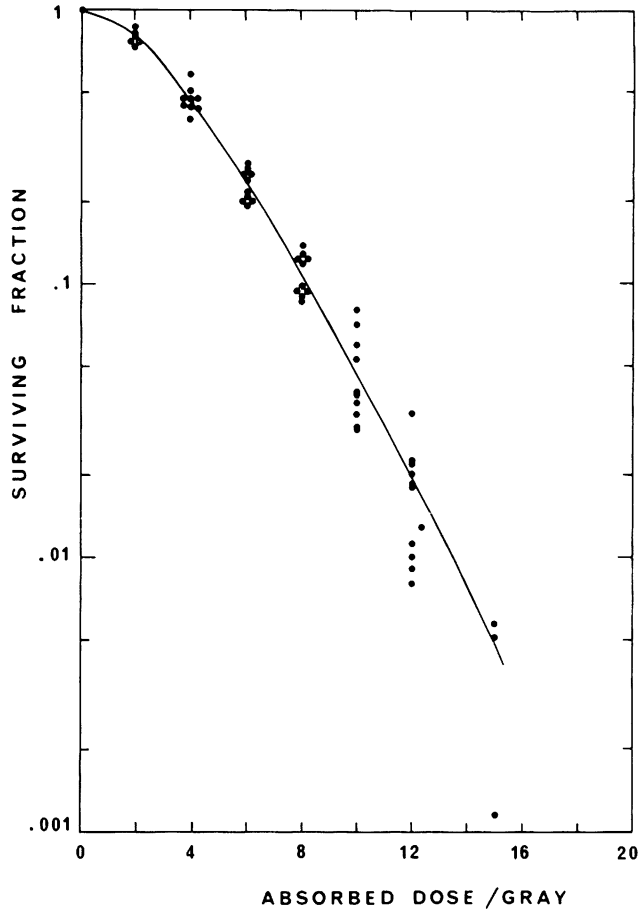

FIG. 4. The uncorrected data from Fig. 2 fitted to one survival curve without dose-modifying factors.

clarity of representation, data points belonging to the different experiments are not distinguished. However, even in the simplified representation one recognizes that the overall fit is adequate and that possible differences in the *shape* of the survival curves must be minor.

The analysis supports the earlier conclusion that sizable fluctuations of sensitivity occur when survival curves are taken in separate experiments under seemingly identical conditions. The cause of these variations has not been identified, but their presence clearly indicates the possible influence of interexperimental variations in experimental studies that involve the comparison of survival curves. The curve in Fig. 3 can be considered the best estimate of the ideal survival curve for V-79 hamster cells exposed to X rays. It has the advantage of being based on the total information from all 10 experiments and of not being biased by the assumption of any particular analytical formula. However, the estimated curve is reliable only for the range of doses with actual observations. The extrapolation to other doses must remain arbitrary. In particular, one can make no definite statement on the

precise value of the initial slope of the curve, as no data were obtained for doses below 2 Gy. On the other hand, one can readily identify a range of possible values of the initial slope.

Figure 4 gives, for comparison, the data in the original form without dose-modifying factors. This corresponds to a straightforward superposition of all data. The solid curve is the nonparametric fit that results when all data are considered as part of one single experiment. The resulting curve is, in this particular case, similar to the curve obtained by the more sophisticated fitting procedure. Nevertheless, there are differences between the two resulting curves; thus the curve in Fig. 4 is nearly straight at large doses, while the curve in Fig. 3 continues to bend over.

The example of a set of relatively well defined X-ray survival curves has been considered here because unavoidable fluctuations in the repeated determination of a survival curve are a problem of considerable radiobiological interest, and it seemed desirable to reexamine the result of the earlier analysis. However, as pointed out, the method of nonparametric curve fitting may be of still greater importance if one deals with poorly defined survival curves, i.e., with greater statistical errors. In such cases an objective algorithm will be required because any curve fitting by hand could be highly arbitrary.

CONCLUSION

The numerical method that has been described permits the construction of theoretical survival curves from experimental data without the need of postulating analytical expressions that are assumed to fit the data. A simpler and more general condition, such as convexity (from above) in the semilogarithmic representation, is sufficient.

The method is readily applicable to individual survival curves, or other dose–effect relations. It can be of special value in those cases where it is desirable to represent a series of slightly differing survival curves by one common theoretical curve and a set of dose-modifying factors.

APPENDIXES

A. Relation between the Standard Deviation of the Survival and the Standard Deviation of the Logarithm of Survival

The standard error σ_s of a survival ratio experimentally obtained at a certain dose can be estimated in the usual way. If I vials are exposed, if these yield the survivals s_i ($i = 1, 2, \ldots, I$), and if $\bar{s}$ is the mean of s_i then

$$\sigma_s^2 = \frac{1}{I(I-1)} \sum_{i=1}^{I} (s_i - \bar{s})^2. \tag{A1}$$

If only one vial is exposed one must set $\bar{s}$ equal to the observed value s, and one can estimate the standard deviation on the basis of Poisson statistics. If N is the estimated number of plated cells and n is the number of viable colonies then

$$\sigma_{n/N}^2 = \sigma_n^2/N^2 = n/N^2. \tag{A2}$$

With $s = n/N$ one therefore obtains

$$\sigma_s^2 = s^2/n. \tag{A3}$$

The standard deviations estimated on the basis of Poisson statistics are somewhat smaller than the actual deviations that may partly be due to factors other than the limited cell number. In one experimental investigation (*5*), where a comparison of the two standard deviations has been performed, the difference was less than 20%.

The standard error of the logarithm of survival can be linked to the standard error of survival. This can be seen in the following way:

$$\sigma_l^2 = \overline{(\ln(s) - \overline{\ln(s)})^2}. \tag{A4}$$

If the fluctuations are small ($|(s - \bar{s})/\bar{s}| \ll 1$) one has the approximate relation

$$\ln(s) - \overline{\ln(s)} \approx \ln(s/\bar{s}) \approx (s - \bar{s})/\bar{s}. \tag{A5}$$

Therefore

$$\sigma_l^2 \approx \overline{(s - \bar{s})^2}/\bar{s}^2 = \sigma_s^2/\bar{s}^2. \tag{A6}$$

Alternatively one can estimate σ_l^2 directly from the values t_i ($i = 1, 2, \ldots, I$) obtained for N vials that are exposed to the same dose.

If the standard deviation is estimated on the basis of Poisson statistics one obtains [see Eqs. (A3) and (A6)]

$$\sigma_l^2 \approx \sigma_s^2/\bar{s}^2 = 1/n; \tag{A7}$$

i.e., the estimated standard deviation of the logarithm of survival is equal to the inverse square root of the number of viable colonies.

B. Maximum Likelihood and Least-Squares Criterion

In cell survival experiments one usually deals with large numbers of plated cells and large numbers of viable colonies. In other experiments, however, the number of irradiated units per dose and/or the number of units that show the effect can be small. Induction of certain mutations or cell transformations, or survival studies on higher animals are examples. In these cases the least-squares criterion may not be valid; one can instead use the maximum likelihood condition. Let N_i be the number of irradiated units at dose D_i, and n_i the number of units that show the effect. Furthermore let S_i be the theoretical probability that an irradiated unit will not show the effect at dose D_i. The maximum likelihood condition is then

$$L = \prod_i \binom{N_i}{n_i} (1 - S_i)^{n_i} S_i^{N_i - n_i} = \text{Max}. \tag{A8}$$

This is equivalent to the condition

$$\phi = -\sum_i (n_i \ln(1 - S_i) + (N_i - n_i) \ln(S_i)) = \text{Min}; \tag{A9}$$

ϕ can be used as the economical function in the optimization procedure.

If the number of exposed units is large while the number of affected units is small, one can use Poisson probabilities instead of binomial probabilities. Instead of Eq. (A8) one then obtains

$$L = \prod_i e^{-(1-S_i)N_i}(1 - S_i)^{n_i}N_i^{n_i}/n_i! = \text{Max.} \tag{A10}$$

The economical function can therefore be chosen as

$$\phi = -\sum_i (n_i \ln (1 - S_i) + N_iS_i) = \text{Min.} \tag{A11}$$

If the number of affected units is large, the maximum likelihood condition reduces to the least-squares criterion. This can be seen in the following way. If the number of unaffected as well as the number of affected units is large, one can write the maximum likelihood condition in terms of normal distributions:

$$L = \prod_i \frac{1}{\sigma_i(2\pi)^{1/2}} e^{-(s_i-S_i)^2/2\sigma_{s_i}^2}. \tag{A12}$$

This is equivalent to the least-squares criterion for the survivals:

$$\phi = \sum_i (s_i - S_i)^2/\sigma_{s_i}^2 = \text{Min.} \tag{A13}$$

The least-squares criterion for the survivals is equivalent to that for the logarithms of the survivals, provided the relative fluctuations are small ($|(s - \bar{s})/\bar{s}| < 1$). In this case one has

$$(s_i - S_i)/S_i = \ln (s_i) - \ln (S_i) \tag{A14}$$

and accordingly, if S_i is set equal to $\bar{s}$ in Eq. (A6):

$$\phi = \sum_i \frac{(s_i - S_i)^2}{\sigma_{s_i}^2} = \frac{(\ln (s_i) - \ln (S_i))^2}{\sigma_{t_i}^2} = \sum_i \frac{(t_i - T_i)^2}{\sigma_{t_i}^2} = \text{Min.} \tag{A15}$$

C. Formulas for the Subroutines that are Required for Fitting a Survival Curve

The formula for the economical function $\phi(T_i)$ is given in Eq. (3). The formula for the constraints C_i is given in Eq. (5). The partial derivatives of the economical function are

$$\frac{\partial\phi(T_i)}{\partial T_i} = 2\sum_{i=1}^{I} (T_i - t_i)/\sigma_i^2. \tag{A16}$$

The partial derivatives of the constraints are

$$\begin{aligned} \partial C_i/\partial T_k &= D_{i+2} - D_{i+1} && \text{for } k = i \\ &= -D_{i+2} + D_i && \text{for } k = i + 1 \\ &= D_{i+1} - D_i && \text{for } k = i + 2 \\ &= 0 && \text{otherwise.} \end{aligned} \tag{A17}$$

D. Equations for the Subroutines in the Joint Fitting of Survival Curves that Differ by Dose-Modifying Factors

It is assumed that the survival curves k ($k = 1, 2, \ldots, K$) differ by dose-modifying factors f_k. The theoretical function, logarithm of survival, $T(d)$, that depends on the "effective dose", d, is to be estimated on the basis of the experimental data. The effective dose, d, takes the values $f_k \cdot D_{ik}$. The optimization procedure determines the values T_n of the function $T(d)$ on the equidistant grid of values $d = n \cdot \Delta$ ($n = 0, 1, \ldots, N$). In survival studies a suitable value for Δ will usually be between 0.1 and 1 Gy, and usually N need not be larger than 50. Values of $T(d)$ for arguments d not on the grid points are obtained by linear interpolation between the values $T_n = T(n \cdot \Delta)$.

The N values T_n and the K dose-modifying factors f_k are to be determined. In addition modifying factors $\exp(g_k)$ are assigned to the different survival curves. These factors correspond to vertical shifts g_k, i.e., to shifts in the logarithm of survival; they account for differences in the plating efficiency between experiments that lead to the individual survival curves.

f_1 may be set equal to 1, and g_1 may be set equal to 0. Furthermore, the values g_k have to be equal for those survival curves that share the same controls. This can be accounted for by stating appropriate ranges for the variables, by adding equations as additional constraints, or by minor modifications of the formulas given below.

The least-squares condition provides the equation for the economical function

$$\phi(T_1, \ldots, T_N, f_1, \ldots, f_K, g_1, \ldots, g_K) = \sum_{i,k} (t_{ik} + g_k - (1 - p_{ik})T_{n_{ik}} - p_{ik}T_{n_{ik}+1})^2 = \text{Min} \quad \text{(A18)}$$

with

$$\begin{aligned} n_{ik} &= \text{Int}(f_k \cdot D_{ik}/\Delta), \\ p_{ik} &= \text{Frac}(f_k \cdot D_{ik}/\Delta). \end{aligned} \quad \text{(A19)}$$

$\text{Int}(\cdot)$ designates the closest integer value below or equal to $f_k \cdot D_{ik}/\Delta$, and $\text{Frac}(\cdot)$ designates the remaining fractional part of $f_k \cdot D_{ik}/\Delta$.

The partial derivatives of the economical function are

$$\frac{\partial \phi}{\partial T_\nu} = -\sum\nolimits_1 2(1 - p_{ik})(t_{ik} + g_k - (1 - p_{ik})T_{n_{ik}} - p_{ik}T_{n_{ik}+1}) - \sum\nolimits_2 2p_{ik}(t_{ik} + g_k - (1 - p_{ik})T_{n_{ik}} - p_{ik}T_{n_{ik}+1}), \quad \text{(A20)}$$

Sum 1 extends over all i,k with $n_{ik} = \nu$,
Sum 2 extends over all i,k with $n_{ik} + 1 = \nu$.

The partial derivatives $\partial\phi/\partial T_\nu$ have discontinuities whenever $f_k \cdot D_{ik}$ is a multiple of Δ, i.e., if $f_k \cdot D_{ik}$ lies on a grid point. However, this has not caused difficulties with the convergence of the optimization algorithms.

$$\frac{\partial \phi}{\partial f_k} = \sum_{i=1}^{I_k} 2D_{ik}/\Delta(T_{n_{ik}} - T_{n_{ik}+1}) \times (t_{ik} + g_k - (1 - p_{ik})T_{n_{ik}} - p_{ik}T_{n_{ik}+1}), \qquad k = 1, \ldots, K,$$
$$\frac{\partial \phi}{\partial g_k} = \sum_{i=1}^{I_k} 2(t_{ik} + g_k - (1 - p_{ik})T_{n_{ik}} - p_{ik}T_{n_{ik}+1}), \qquad k = 1, \ldots, K. \tag{A21}$$

The constraints are

$$C_\nu = T_\nu - 2 \cdot T_{\nu+1} + T_{\nu+2} \leq 0, \qquad \nu = 1, \ldots, N - 2. \tag{A22}$$

The partial derivatives of the constraints are

$$\begin{aligned} \partial C_\nu/\partial T_\mu &= 1 \qquad \text{for } \mu = \nu \quad \text{and} \quad \mu = \nu + 2 \\ &= -2 \qquad \text{for } \mu = \nu + 1, \\ &= 0 \qquad \text{otherwise}, \\ \nu &= 1, \ldots, N - 2, \qquad \mu = 1, \ldots, N. \end{aligned} \tag{A23}$$

E. Analysis of Errors

In the conventional fitting of survival curves by analytical expressions with specified numbers of free parameters, one can calculate confidence ranges of the parameters [see, for example, (*10*, *11*)]. In the present case numerous parameters are estimated that are coupled by constraints. It is therefore uncertain whether an effective number of free parameters can be assigned to the fitting procedure, or whether the concept of free parameters is applicable at all. It is possible, however, to derive confidence intervals for the dose-modifying factors. Such confidence intervals are given in Table I; their derivation will be considered in the following.

Assume that a sufficiently large number of survival experiments are fitted simultaneously. The resulting shape of the survival curve will then not substantially change if the fit is performed with the exclusion of one of the experiments. Within this approximation it is then justifiable to disregard the correlation between the estimated dose-modifying factor f_k for one experiment and the estimated shape of the survival curve. The estimated value f_k is associated with the least sum of squares ϕ_k for curve k. The terminal points of the 95% confidence range of f_k correspond then to increased values $\phi_k + \Delta\phi$ that are determined according to the Fisher distribution (*10*, *11*):

$$\Delta\phi = \phi_k \cdot M/(N - M) \cdot F(M, N - M, \alpha). \tag{A24}$$

In the present case $M = 1$ since one deals with only one free parameter f_k. N is the number of data points for the experiment k (excluding the control). The statistical level α is, in the present calculation, set equal to 95%.

A more rigorous analysis includes not only the horizontal shift f_k, but also the vertical shift factor. The formula is then slightly modified. In the present case the vertical shift factors are close to unity, and their influence can be neglected.

In fact, both methods of calculation lead to nearly the same numerical values, and the simpler method is therefore presented.

Equation (A24) serves to determine the critical values $\phi_k + \Delta\phi$ of the sum of squares. The functional dependence between f_k and the sum of squares is evaluated numerically for each of the survival curves; this leads to the confidence ranges given in Table I.

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