Biological Complexity and Radiosensitivity

Radiation lethality in cells and viruses is correlated with nucleic acid content, structure, and ploidy.

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A relationship between the size of viruses and their sensitivity to inactivation by ionizing radiation has long been recognized (*1*). Indeed, such data constituted a substantial part of the evidence adduced in support of target theory (*2*). Later investigations indicated that a better correlation obtains between radiosensitivity and DNA or RNA content than between radiosensitivity and virus size (*3*), and it has been suggested (*4*) that a similar correlation may also hold for microbial and animal cells. The radiosensitivity of plant systems has also been shown to be closely correlated with nuclear size, ploidy, and DNA content (*5*).

Terzi (*6*) reviewed a large body of literature relating to (i) radiosensitivity and (ii) nucleic acid content in a number of viruses and cells. The index of radiosensitivity he employed was the x-ray dose (D_0 or D_{37}), in roentgens, corresponding to a surviving fraction of e^{-1}, or 0.37, estimated from the slope of the exponential portion of dose-survival curves for cells or viruses irradiated under "direct effect" conditions. Survival was equated with the capacity to replicate, expressed in terms of plaque formation in the case of viruses and of colony (clone) formation in the case of microbial, avian, and mammalian cells. Nucleic acid content (RNA content for RNA viruses, DNA content for all other entities) was given in molecular weight units. Terzi found that "the lethal efficiency (e) per ion pair produced in the nucleic acids" tended to have different characteristic values in four groups of biological entities: RNA and single-stranded DNA viruses (average $e = .64$); double-stranded DNA viruses (average $e = .62 \times 10^{-1}$); haploid bacteria and yeast (average $e = 1.3 \times 10^{-2}$); mammalian and avian cells and diploid yeast (average $e = .69 \times 10^{-3}$).

The analysis which we present here was initiated independently of Terzi's work; the same indices were used, except that RNA or DNA content was expressed in terms of nucleotide content, 300 being taken as the average molecular weight per nucleotide. Our analysis may be viewed as building on Terzi's observations in two ways: first, additional data are brought in and some of Terzi's data are critically reconsidered (Table 1); secondly, the data are subjected to a more refined mathematical treatment, which produces some interesting tentative hypotheses. Despite recent modifications in target theory (*7*), ambiguities and pitfalls of interpretation persist, as Zimmer (*8*) has stressed. Although no definitive conclusions are yet possible, it is hoped that presentation of the material in this form will stimulate efforts to obtain additional reliable data on these parameters for a sufficient number and variety of biological entities to permit critical evaluation of these and other possible models of radiobiological action.

In Fig. 1 are displayed data points representing various cells and viruses of the four designated classes. Each point has two coordinates, the horizontal being the logarithm of the dose producing 37-percent survival (D_{37}) and the vertical being the logarithm of the DNA nucleotide content of the cell or virus (or of the RNA nucleotide content for RNA viruses). Terzi's conclusion can be interpreted in terms of this graph in the following way. First, one observes from study of his Table 1 (*6*) that

$$e = \frac{3.7 \times 10^{11}}{D\,N} \qquad (1)$$

where D is the dose (in roentgens) producing 37-percent survival and N is the nucleic acid content in molecular weight units. Taking logarithms and rearranging Eq. 1, we obtain

$$\log D + \log N = 11.57 - \log e. \qquad (2)$$

The observation that e (and thus log e) tends to have four different values, each fitting well all the points in its group, means that all the points in a group lie reasonably close to a straight line (relating log D and log N) of the form of Eq. 2, and that the four lines are quite distinct. In Fig. 1, those four lines (each with slope −45 degrees because of Eq. 2) are shown; each line passes through its group average in both coordinates, and indeed does lie close to the points of its group and away from the other lines.

But one is struck by the fact that in three of the groups the data suggest a straight line with a slope definitely steeper than 45 degrees. In the fourth group (haploid bacteria and yeast) no clear pattern is seen, because of the short range in both variables. Further analysis of these apparent straight lines is needed.

There is a little ambiguity about how one should estimate the slope of a straight line when both variables contain random error. This is surely the case here: both N [nucleotide content (*9*)] and D (dose for 37-percent survival) have random error. This is larger in the case of D, so D has been chosen as the "dependent variable" in the analysis (*10*). The findings are qualitatively about the same no matter how the fitting is done, but all tests of significance are a bit approximate. Nonetheless, fitting four straight lines gives the following estimated slopes and (in parentheses) standard errors of those slopes.

RNA and single-stranded DNA viruses, − .65 (.08)
Double-stranded DNA viruses, − .81 (.13)
Haploid cells, − .13 (.53)
Diploid cells, − .85 (.04).

These values rather clearly require rejection of the notion of a common slope of −1.0, corresponding to a −45-degree line. It was then asked whether the four sets of data are compatible

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with four straight lines having different intercepts but *some* one common slope (which would clearly have to have a value around −.7 or −.8). The least-squares method gives an estimated value for such a common slope (*10*) of −.809, with a standard error of .036. Whether fitting four lines with this slope is straining too hard can be tested (approximately) by means of an F-test having 3 and 23 degrees of freedom. The obtained value is 1.94, which is not significant, even at the 10-percent level. This means that fitting parallel lines gives a fit not significantly worse than that obtained by using, for each line, its own best slope. Thus we may, with a little diffidence, adopt this simple representation and undertake interpretation of the following summary of the data:

RNA and single-stranded DNA viruses:
$$\log D + .809 \log N - 8.65 = 0 \quad (3a)$$
Double-stranded DNA viruses:
$$\log D + .809 \log N - 9.32 = 0 \quad (3b)$$
Haploid cells:
$$\log D + .809 \log N - 9.58 = 0 \quad (3c)$$
Diploid cells:
$$\log D + .809 \log N - 10.29 = 0. \quad (3d)$$

These lines, together with the data points, are shown in Fig. 2. It is to be noted that the expressions 3*a*–3*d* resemble Eq. 2, except that in Eq. 2 the coefficient of log N is 1.0, implying a −45-degree line. Formulas 3*a*–3*d* can be summarized in one formula for $i = 1,2,3,4$, as

$$\log D_{37} + .809 \log N + \log \alpha_i = 0. \quad (4)$$

Now we write the equation of which Eq. 4 is the logarithm:

$$\alpha_i D_{37} N^{.809} = 1. \quad (5)$$

We multiply through by −1 and again take exponentials, obtaining

$$\exp(-\alpha_i D_{37} N^{.809}) = \exp(-1). \quad (6)$$

We now undertake to present a theoretical model in terms of which Eq. 6 can be interpreted.

We begin by focusing on a very small part of the total nucleotide complement of a cell. Let us call this small part dN. How can radiation cause reproductive failure of the whole cell by its action on this small fragment? First, the fragment must be "hit"; the probability that it will be is proportional to D, the flux of quanta, and to the volume of dN. We write this probability (which is very small indeed, if dN is) as $CDdN$, where C is a constant not depending on N or D. Second, the hit must result in reproductive failure of the whole cell; we write this probability as the product of two components (*11*) —one, ξ_i, depending on the *kind* of cell (haploid, diploid, and so on), the other, φ_N, depending on the nucleotide content. Thus we propose:

$$P\left\{\begin{array}{l}\text{that, a hit having occurred}\\ \text{in } dN\text{, reproductive}\\ \text{failure follows}\end{array}\right\} = \xi_i \varphi_N. \quad (7)$$

Then the probability (P) that there is a hit in dN, and that reproductive failure follows is

$$\xi_i \cdot \varphi_N \cdot C \cdot D \cdot dN. \quad (8)$$

If we then assume that φ_N is the same in every fragment dN, and that survival follows only if every small fragment dN fails to be the site of a hit causing reproductive failure (*12*), we find, from the standard theory of the Poisson process, that

$$P \text{ (survival)} = \exp(-\xi_i \varphi_N CDN) \quad (9)$$

and at D_{37}, by definition, Eq. 9 has the form

$$\exp(-1) = \exp(-\xi_i \varphi_N C D_{37} N). \quad (10)$$

We now compare Eq. 6 with Eq. 10 and deduce

$$\alpha_i N^{.809} D_{37} = \xi_i C \varphi_N N D_{37}. \quad (11)$$

This equality leads to the following identifications

$$C\xi_i = \alpha_i \quad (12)$$

and

$$\varphi_N = N^{-.191}$$

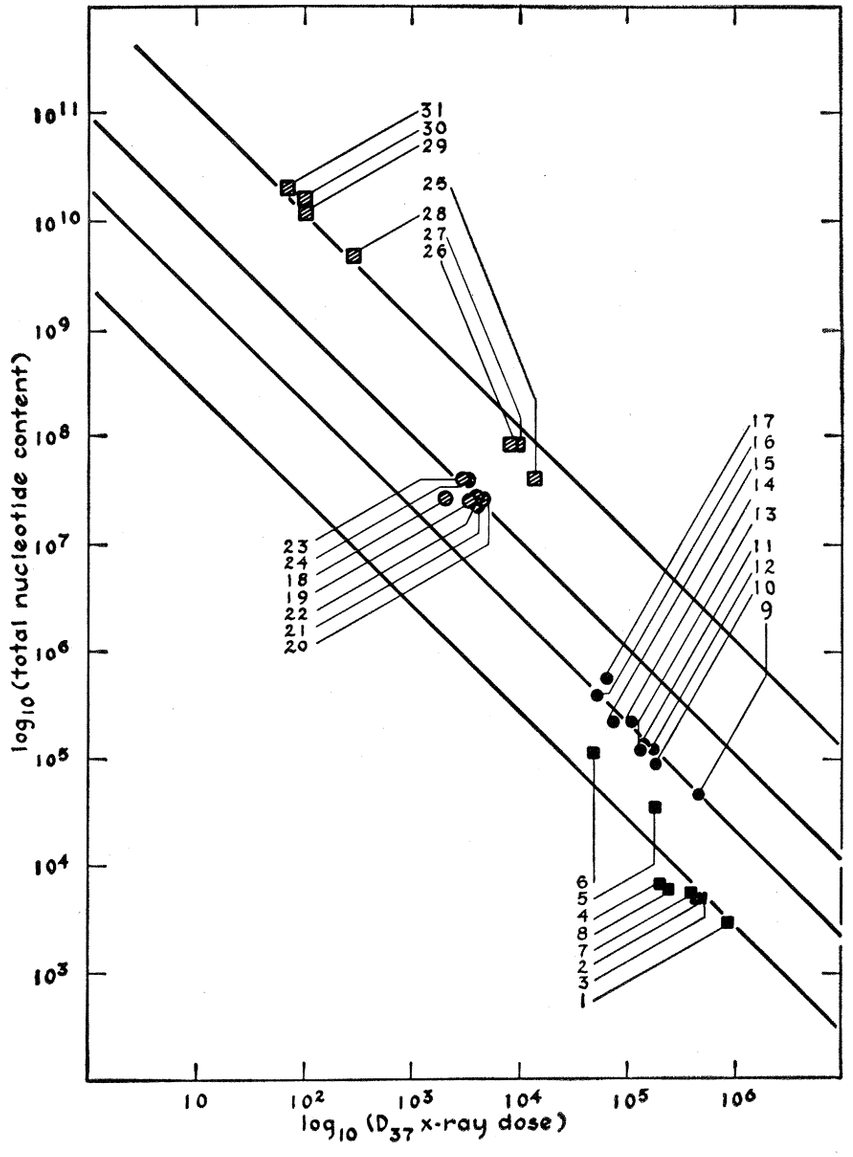

Fig. 1. Forty-five-degree lines fitted to data points representing various cells and viruses of four classes: (solid squares) RNA and single-stranded DNA viruses; (solid circles) double-stranded DNA viruses; (hatched circles) haploid bacteria and yeast; (hatched squares) mammalian and avian cells and diploid yeast.

or, more generally,

$$\varphi_N = N^{\beta-1} \qquad (13)$$

At this point we see that α_i involves two parts; C is the proportionality constant relating the product of dose and volume to the probability of a hit and may be thought of as reflecting the probability of absorption by DNA, while ξ_i has to do with the probability that such a hit will, in a cell of type i, result in reproductive failure. The value for C can possibly be estimated, but in comparing the various lines this value is irrelevant since

$$\log \alpha_i = \log C + \log \xi_i,$$

and in differences among the log α_i the log C components cancel each other.

It should be noted that if the family of straight lines had been parallel, with slope $= -1$ (corresponding to -45-degree lines), then .809 would be replaced by 1 in Eq. 6, and φ_N in Eq. 13 would be $N^\circ = 1$. Thus the factor φ_N arises from the fact that the straight lines are not 45-degree lines.

From Eqs. 12 and 13 we know that

$$\xi_i \varphi_N = (\alpha_i/C) N^{\beta-1} \qquad (14)$$

denotes the probability that a hit which has occurred at a given point will result in reproductive failure.

At least two ways of interpreting α_i/C and $N^{\beta-1}$ occur to us.

1) α_i/C represents the probability that a hit at a point in the nucleic acid of a cell of type i, of any size, effects a genetic change, and $N^{\beta-1}$ represents the probability that the genetic change then inactivates reproduction. In this interpretation $N^{\beta-1}$ is a measure of the *fraction* of genetically controlled phenotypic characters which are essential to reproductive life. From this interpretation it follows that the greater the nucleotide content, the smaller the *fraction of it* which is essential to reproduction.

2) α_i/C represents the probability that a hit at a point in the nucleic acid of a cell of type i inactivates a reproductively essential feature of the cell (here again, the probability is assumed to be the same for large as for small cells); $N^{\beta-1}$ represents the probability that the damage is left unrepaired by the cell. Thus, the larger the cell, the more likely it is to arrange the repair of such an injury. (But as N grows, this ability grows less rapidly than does the total number of hits to be repaired, so radiosensitivity increases with the amount of nucleotide.)

It should be remarked that, in both interpretations, α_i/C is independent of the size (N) of the cell but does depend upon its type (haploid, diploid, and so on). Conversely, φ_N is independent of the type of cell in both models 1 and 2.

Finally, mixtures between models 1 and 2 are possible in the sense that model 1 might hold and φ_N be a product of two quantities $\varphi_{1,N}$ and $\varphi_{2,N}$, one referring to the fraction of loci essential for reproduction, the other referring to the probability of repair.

It remains to give an interpretation of the differences among the intercepts. Let α_i^* denote the intercept for the line representing cells of type i; then $\alpha_i^* = \log \alpha_i$ in the foregoing discussion. The estimates for α_i^* are displayed in Eqs. 3*a*–3*d*. All the differences among them are statistically significant beyond the .001 level, except that

$$\alpha_2^* - \alpha_3^* = -.261$$

has a standard error of .098 and is significantly different from zero at only about the 1-percent level. The four values of α_i^* decline as i represents, first, RNA and single-stranded DNA viruses, then double-stranded DNA viruses, then haploid bacteria and yeast, then m-ploid ($m = 2$) cells; this decline means *decreasing* radiosensitivity (for fixed nucleotide content) as one moves

Table 1. Nucleotide content and radiosensitivity of various cells and viruses.

Entity	Code No.	Nucleotides per genome*	Ref.	D_{37} (rad)†	Ref.	Comment
		Group A_1: RNA viruses				
Phage R17	1	3.0×10^3	(*7*)	8.4×10^5	(*8*)	‡
Tobacco ringspot virus	2	5.0×10^3	(*19*)	4.6×10^5	(*20*)	
Tomato bushy stunt virus	3	5.0×10^3	(*21*)	4.5×10^5	(*20*)	
Tobacco mosaic virus	4	$6.5–7.2 \times 10^3$	(*19, 22*)	2.0×10^5	(*23*)	§
Rous sarcoma virus	5	$3.2–4.0 \times 10^4$	(*24*)	$1.6–2.0 \times 10^5$	(*25*)	‡
Newcastle disease virus	6	1.1×10^5	(*19*)	4.6×10^4	(*26*)	‡
		Group A_2: DNA viruses (single-stranded)				
Phage φX174	7	5.5×10^3	(*27*)	3.8×10^5	(*28*)	§
Phage S13	8	6.0×10^3	(*29*)	$2.3–2.5 \times 10^5$	(*30*)	
		Group B: DNA viruses (double-stranded)				
Shope papilloma virus	9	4.7×10^4	(*31*)	4.4×10^5	(*32*)	
Phage BM	10	8.3×10^4	(*33*)	1.9×10^5	(*34*)	
Phage T7	11	$1.2–1.6 \times 10^5$	(*35*)	1.35×10^5	(*28*)	‖, §
Phage T1	12	1.3×10^5	(*36*)	1.7×10^5	(*28*)	‖
Phage P22	13	1.3×10^5	(*36*)	1.25×10^5	(*37*)	
Phage λ	14	2.3×10^5	(*38*)	1.0×10^5	(*39*)	
Adenovirus, type V	15	2.2×10^5	(*40*)	7.0×10^4	(*41*)	‡
Phages T2, T4	16	4.3×10^5	(*42*)	$5.0–5.2 \times 10^4$	(*43*)	‖, ¶
Vaccinia virus	17	5.2×10^5	(*40*)	$4–10 \times 10^4$	(*44*)	‖, §
		Group C: haploid microorganisms				
Escherichia coli						
Strain B	18	$2.3–3.0 \times 10^7$	(*45*)	2.0×10^3	(*46*)	‡
Strain H	19			3.3×10^3	(*47*)	
Strain B/r	20			4.5×10^3	(*48*)	
Bacterium aertrycke	21	2.4×10^7	(*49*)	4.0×10^3	(*50*)	
Micrococcus pyogenes var. *aureus*	22	2.8×10^7	(*49*)	4.0×10^3	(*51*)	
Haemophilus influenzae	23	4.0×10^7	(*52*)	3.6×10^3	(*52*)	#, ¶
Saccharomyces cerevisiae	24	4.0×10^7	(*53*)	$2.8–3.7 \times 10^3$	(*54*)	#
		Group D: diploid cells				
Diplococcus pneumoniae	25	4×10^7	(*52*)	1.3×10^4	(*55*)	‡
Escherichia coli Strain P-6	26	$8–9 \times 10^7$	(*56*)	8.5×10^3	(*56*)	‡
Saccharomyces cerevisiae	27	$8–9 \times 10^7$	(*53*)	$7–13 \times 10^3$	(*54*)	¶
Chicken embryo cells	28	5×10^9	(*57*)	$2.7–3.2 \times 10^2$	(*58*)	§
Mouse (bone marrow, in vivo)	29	1.3×10^{10}	(*57*)	1.1×10^2	(*59*)	‡; DNA data for rat
Guinea pig	30	1.7×10^{10}	(*57*)	1.0×10^2	(*60*)	
Human (fibroblasts)	31	2.1×10^{10}	(*57*)	$0.5–1.0 \times 10^2$	(*15*)	

* DNA content per cell or infectious particle (RNA content in the case of RNA viruses) converted to molecular weight units and divided by 300.

† D_{37}: the radiation dose at which the surviving fraction is $1/e = 0.37$. Where this value is not explicitly stated in a cited reference, it has been estimated from the exponential portion of the dose-log survival curve presented in the publication cited.

‡ Not included in Terzi's compilation. Fowl plague virus with phages T5 and P-8, listed by Terzi, were omitted here after careful review of the original sources indicated that the radiation or nucleic acid data, or both, were probably unreliable. Terzi also cites data for DNA content (*52*) and radiosensitivity (Drew, 1955) of *Diplococcus pneumoniae*. Although the corrected values for nucleotide content (4×10^7) and for the D_{37} (1.3×10^4 rep) which we derive from these sources yield a point on the diploid-cell isosensitivity line of Fig. 2, we are not entirely certain that this organism should be regarded as diploid.

§ Radiation data cited by Terzi have been superseded by data of the reference cited.

‖ Nucleic acid data cited by Terzi have been superseded by data of the reference cited.

¶ Radiation data are incorrectly cited by Terzi from this reference.

\# Nucleic acid data are incorrectly cited by Terzi from this reference.

through this set of cell types in the same order.

If scission of the nucleic acid sugar-phosphate ester backbone is the major radiobiochemical lesion leading to impairment of reproductive capacity, then it is readily understandable that the probability of breaking *both* strands in double-stranded DNA viruses would be appreciably smaller than the probability of breaking a single strand in phages φX174 and S13, and in the RNA viruses. At the cellular level, the probability of occurrence of recessive lethals would be much smaller in diploid than in haploid cells, although this would be partly compensated for by the added possibility of dominant lethal events.

Now (if C is disregarded), α_i (under model 1) denotes the probability that a mutation results from a hit, or (under model 2) the probability that a mutation which inactivates a *reproductively essential* feature of the cell results. We now write these probabilities in terms of genetic burden and breakage,

$$\alpha_1 = b + \lambda + \rho \quad (15)$$

$$\alpha_2 = \alpha_3 = \lambda + \rho \quad (16)$$

$$\alpha_4 = \lambda + \mu. \quad (17)$$

In these expressions, λ denotes the probability that a hit is on a dominant locus and effects a lethal mutation (*13*); ρ denotes the probability (in either of the first two cell types) that a hit is on a recessive locus and effects a lethal mutation; μ denotes the probability (in a diploid) that the hit is on the second of two homologous recessive loci, the first already having been hit (*14*); b denotes the probability of a hit's breaking a single strand.

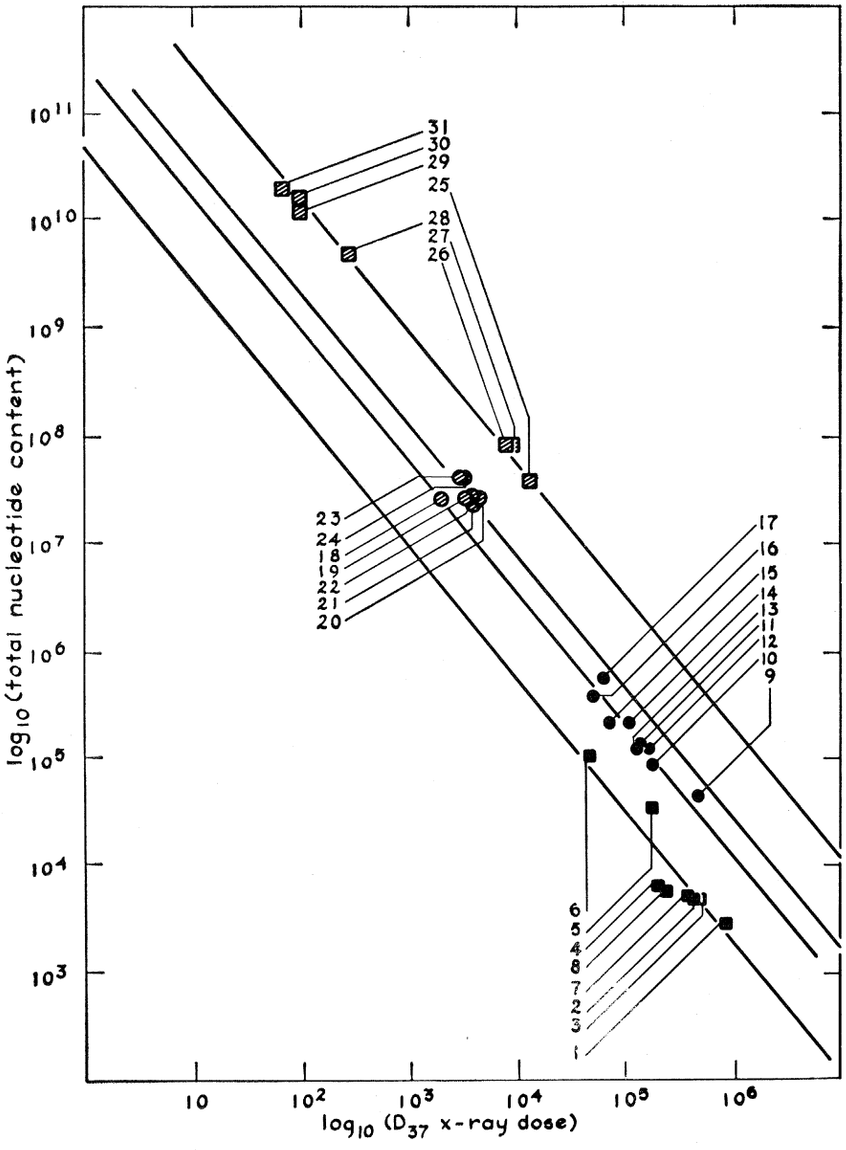

Fig. 2. Lines corresponding to Eqs. 3*a*–3*d*, with corresponding data points.

Study of Eqs. 15, 16, and 17 shows that $\alpha_1 > \alpha_2 = \alpha_3 > \alpha_4$ is to be expected, since $b > 0$ and $\rho > \mu$.

The entire discussion to this point has been in terms of parallel straight lines. It is possible to generalize the model to nonparallel straight lines, which, as noted, give a slightly better fit. However, the analysis and interpretation are much more complex and less intuitively appealing.

Additional analytical data on DNA or RNA content and on radiosensitivity (scored in terms of reproductive death) for a variety of other cells and viruses will be required to further test these hypotheses. Recent data for radiation-induced lethality in certain plant cells (*15*) seem reasonably consistent with expected findings for diploid cells of corresponding DNA content. In contrast, the anomalously high radioresistance of certain microorganisms (*16*) remains to be reconciled in molecular terms with the normal pattern of radiation responsiveness. The recent discovery that the RNA of certain viruses is double-stranded (*17*) makes available for the first time a biological system in which the radiosensitivity of double-stranded RNA may be compared with the established response of double-stranded DNA. Finally, it is to be hoped that, as additional data of sufficiently high precision become available, it may become possible to refine this type of analysis to take into account the influence of other parameters, such as base composition (*18*).

Appendix

As noted, it has been assumed that reproductive survival requires the absence of a single (unrepaired) hit on any reproductively essential site. This is a one-hit model. If a k-hit model is introduced, then (i) the estimated lines are not changed (this is natural since they are merely a summary of the data, not consequences of the model); (ii) $N^{\beta-1}$ remains unaltered; (iii) the interpretation of α_i is modified. Verification of points ii and iii proceeds along the following lines. Equations 4, 5, and 6 are unaffected. The development beginning at Eq. 7 is somewhat altered. If hits on k (or more) reproductively essential targets are required for reproductive failure, then we write

$$P\left\{\begin{array}{l}\text{that a hit which occurred}\\ \text{in } dN \text{ inactivates a repro-}\\ \text{ductively essential target}\end{array}\right\} = \xi_i \varphi_N. \quad (7')$$

Then the probability (P) that there is a hit in dN and that it inactivates a reproductively essential target is

$$\xi_i \cdot \varphi_N \cdot C \cdot D \cdot dN = \lambda dN. \qquad (8')$$

If we then assume that φ_N is the same in every fragment dN, and that survival follows unless k or more fragments dN contain inactivated reproductively essential targets, we find, from the standard theory of the Poisson process, that

$$P(\text{survival}) = \sum_{x=0}^{k-1} \exp\left[-\lambda N\right] \frac{(\lambda N)^x}{x!} = f_k(N\lambda) \qquad (9')$$

where $f_k(\lambda)$ is defined by Eq. 9′ and where λ is as defined in Eq. 8′.

Then at D_{37}, by definition, Eq. 9′ has the form

$$f_k(N\lambda_{37}) = f_k(\xi_i \varphi_N C D_{37} N) = \exp(-1) \qquad (10')$$

whence:

$$\xi_i \varphi_N C D_{37} N = f_k^{-1}[\exp(-1)] = \gamma_k. \qquad (10'')$$

Now, comparing Eq. 5 with Eq. 10″, we have

$$\alpha_i D_{37} N^{.809} = (1/\gamma_k)(\xi_i \varphi_N C D_{37} N). \qquad (11')$$

This equality leads to the following identifications:

$$\alpha_i = \frac{C}{\gamma_k} \xi_i \qquad (12')$$

and

$$\varphi_N = N^{-.191},$$

or, more generally,

$$\varphi_N = N^{\beta-1} \qquad (13')$$

That Eqs. 13 and 13′ are the same verifies point ii. Comparison of Eqs. 12 and 12′ shows that values of α_i/C now relate to ξ_i/γ_k.

Thus, in the k-hit model,

$$\alpha_i^* = \log \alpha_i = \log C + \log \xi_i - \log \gamma_k, \qquad (18)$$

for $k = 1$, $\log \gamma_1 = 0$; for $k = 2$, $\gamma_2 = \log \gamma_k = 1.1461$; and, as k grows, γ_k grows. So as k grows the value of α_i^* drops; this implies an increasing radiosensitivity *per target*, of k necessary targets. This is the modified interpretation of α_i mentioned above (point iii).

References and Notes

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9. Observe that although Terzi's results are in terms of nucleotide mass (N'), we prefer to deal with $N = N' \div$ (average mass for a nucleotide) = nucleotide content. This makes it clear that N is free of the mass units employed—information essential to our later conclusion that N^{-1} may be regarded as a probability.
10. This procedure would be strictly correct if there were *no* error in N. Fitting with N as the dependent variable leads to —.852 rather than —.809; the correct answer would lie between these values, and nearer to the latter, since D has considerably greater variance than N.
11. At this point we do not suppose that there is only one way of doing this; later, we shall see that the character of φ_N does make it unique.
12. Note that we assume here a one-hit theory; in the Appendix the effects of using k-hit theory (for various k) are considered; it is shown that the conclusions are essentially unaffected.
13. Under model 1, this can be any kind of lethal mutation; under model 2 the lethal mutation must inactivate a reproductively essential feature of the cell.
14. The terms μ^2, b^2, and so on, are ignored since they are negligibly small in comparison with μ, b, λ, and ρ.
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