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A Dose–Surviving Fraction Curve for Mouse Colonic Mucosa*

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Abstract—*A dose–surviving fraction curve representing the response of the mouse colonic mucosa to single doses of* ^{137}Cs *gamma radiation was obtained from the results of a multifraction* in vivo *colony assay. Construction of the curve required an estimate of the average number of clonogens initially present per colonic crypt. The estimated clonogen count* (88) *was determined by a statistical method based on the use of doses per fraction common to different fractionation protocols. Parameters for the LQ and TC models of cell survival were obtained by weighted least-squares fits to the data. A comparison of the survival characteristics of cells from the mouse colonic and jejunal crypts suggested that the epithelium of the colon is less radiosensitive than that of the jejunum.*

INTRODUCTION

AN *in vivo* colony assay can be used to measure the acute radiation response of mouse colonic mucosa. Following exposure to radiation, some colonic crypts will be denuded of survivors, while others will contain one or more cells capable of producing colonies. The average number of surviving cells in a cross-section of the colon can be estimated from the observed fraction of repopulated crypts by correcting for the probable multiplicity of survivors in each regenerated crypt. A limitation of this technique is that the radiation dose must be sufficient to depopulate a fraction of the colonic crypts. In particular, the low-dose response cannot be assayed directly.

Multifraction techniques can be used to measure low-dose response provided it is valid to assume that successive radiation doses of equal size reduce the proportion of survivors equally; if so, the response to a single low dose can be inferred from the cumulative response to repeated dose fractions. A dose–survival curve can be constructed if, in addition, it is known how many clonogenic cells are present in each crypt before irradiation. In this paper, we derive an estimate of the average initial clonogen number in the mouse colonic crypt by analyzing previously published data [1]. A dose–survival curve is presented, and parameters for the linear–quadratic (LQ) [2] and two-component (TC) [3] models are obtained.

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MATERIALS AND METHODS

Materials and irradiation procedures

The materials and irradiation procedures have been described previously [1]. Briefly, unanesthetized, pathogen-free C3Hf/Kam mice (previously denoted C3Hf/Bu), 8–12 weeks of age, were exposed to gamma radiation from a ^{137}Cs source situated 28 cm from mid-mouse. Some of the mice received single doses; others were exposed to multifraction regimens in which the number of dose fractions ranged from 2 to 20, given at fixed intervals of 1, 3, 12 or 24 hr. Several days after completion of irradiation, the mice were sacrificed and histological sections of the colon were prepared. The number of crypts that contained colonies of proliferative cells was scored for each section. Two sections per mouse were examined. For consistency in scoring, the time interval between last radiation dose and sacrifice was varied so that the average colony size in each regenerated crypt was kept approximately constant.

Data analysis

Estimate of cell survival. The probability that a colonic crypt contains no surviving clonogens

following irradiation can be approximated by the proportion p of crypts that are denuded [4]. Assuming that cell survival is random, the average number λ of survivors per crypt is related to p by the Poisson equation

$$p = e^{-\lambda}. \quad (1)$$

Since there are, on average, 130 crypts in a cross-section of the mouse colon, average cell survival per crypt can be computed from equation (1) by

$$\lambda = -\ln (1 - Y/130), \quad (2)$$

where Y is the scored number of repopulated crypts. For each fractionation regimen consisting of a dose per fraction x given in n equally spaced fractions, several replicate values for $\lambda = \lambda(n,x)$ of (cumulative) cell survival in the mouse colon were computed using equation (2). Each value of $\lambda(n,x)$ was obtained by taking Y to be the average crypt survival from a pair of scored colonic sections. In most cases the pairs consisted of scores from two sections of the same mouse.

Equal effect per fraction. To infer the average level of cell survival following a single low radiation dose x from the average survival $\lambda(n,x)$ after total dose nx, each of the n dose fractions must reduce survival by the same proportion. In particular, the interval between successive doses must be sufficiently long to allow repair of sublethal injury. The number of fractions must also be small enough that each multifraction regimen is complete before compensatory proliferation begins to occur.

Previous studies have shown that 3-hr fractionation intervals allow essentially complete repair of sublethal injury in the mouse colonic mucosa. It has also been shown [1] that the total dose required in 5 dose fractions to reach an isoeffect of 50 surviving cells per colonic circumference increases only slightly when the fractionation interval is increased from 3 to 12 hr (23.5 Gy for 3-hr intervals vs 25.0 Gy for 12-hr intervals). This suggests that the amount of proliferation occurring during the first 48 hr after onset of irradiation is fairly small. Since this slight proliferation probably occurs near the end of the treatment period, it was reasonable to expect that the colon data from multifraction experiments with 3-hr fractionation intervals and overall time less than 48 hr (i.e. up to 10 or 15 fractions) would satisfy the hypothesis of equal effect per dose fraction.

A statistical method based on the use of common dose fractions [5] was used to test the hypothesis of equal effect per fraction in the mouse colon data with 3-hr fractionation intervals. For each common dose per fraction x given in regimens consisting of different fraction numbers m and n, the average cell survival levels $\lambda(m,x)$ and $\lambda(n,x)$ resulting from the total doses mx and nx were used to compute the quantity $y = n \ln \lambda(m,x) - m \ln \lambda(n,x)$. It has been shown [5] that

$$y = (n - m) \ln K + nm(\mu(m) - \mu(n)), \quad (3)$$

where K is the average number of clonogenic cells initially present per colonic crypt and $\mu(m) - \mu(n)$ is a factor that has the constant value zero only if equal effect per fraction holds. A least-squares fit of the observed quantities $y = n \ln \lambda(m,x) - m \ln \lambda(n,x)$ to the two variables $n - m$ and nm provides a test of the hypothesis of equal effect per fraction; the hypothesis should be rejected if the nm coefficient differs significantly from zero (significance level less than 0.05). Further details of the statistical method can be found in [5] and [6].

Estimate of initial clonogen number. Prior to irradiation each colonic crypt contains an average of K cells capable of producing colonies. An estimate of K is required to determine the mean fraction of surviving cells after radiation exposure from the average per-crypt cell survival λ. Equation (3) shows that an estimate of $\ln K$ is obtained from the regression coefficient of the variable $n - m$ by the statistical procedure described above. However, if the hypothesis of equal effect per fraction cannot be rejected, a better estimate of K is obtained by a regression fit of the observed quantities $y = n \ln \lambda(m,x) - m \ln \lambda(n,x)$ to the single variable $n - m$:

$$y = (n - m) \ln K. \quad (4)$$

RESULTS

The protocols for irradiation of the mouse colon using 3-hr fractionation intervals included eight fraction pairs (n,m) for which a common dose per fraction was administered. The doses common to different regimens are shown in Table 1. The statistical analysis described by equation (3) was performed for the data available from these eight fraction pairs. It was found that the hypothesis of equal effect per fraction could not be rejected for the 3-hr data, as indicated by the regression estimate of the nm coefficient in equation (3) (to three digits, $\mu(m) - \mu(n) = 0.000$, significance level 0.989). This conclusion is valid only for the data from regimens of up to 10 fractions, since 10 is the largest fraction number represented by the common-dose pairs from Table 1.

The possible influence of proliferation in the 15- and 20-fraction data was investigated by plotting reciprocal isoeffect dose vs dose per fraction [7]. The total doses required to reach an isoeffect of 30 regenerated crypts per colonic circumference were determined by logit analysis for fraction numbers ranging from 1 to 20 (Table 2). In Fig. 1 the reciprocal total dose ($1/ED_{50}$) is plotted against dose per fraction (ED_{50}/n) for each fraction number n. The LQ model of cell survival, given by $-\ln(\text{s.f.}) = \alpha(\text{dose}) + \beta(\text{dose})^2$, predicts that these points will lie along a straight line with slope β/E and intercept α/E, provided that equal doses produce equal decrements in cell survival (here E is the log surviving fraction corresponding to the chosen isoeffect). Figure 1 shows the regression line fit to the data corresponding to 10 or fewer fractions. The plot suggests that proliferation is not a significant factor in the 15-fraction data, but that some proliferation probably occurred during the 20-fraction regimens. The point determined by the 20-fraction data is displaced slightly below the line, indicating that the ED_{50} for 20 fractions is somewhat larger than would be predicted by the LQ model alone.

Figure 2 illustrates the method that was used to estimate the average number K of clonogenic cells initially present per colonic crypt. For each of the doses per fraction listed in Table 1 the quantity

Table 1. Doses per fraction common to different fractionation regimens

Dose per fraction (Gy)	No. of fractions					
	2	3	5	6	8	10
4.00				*	*	*
4.20			*			*
4.33			*	*		
4.50			*		*	
6.00			*	*		
9.00	*	*				

Table 2. Doses required for an isoeffect of 30 surviving crypts per colonic circumference using 3-hr fractionation intervals

No. of fractions	Total dose (Gy)	95% confidence interval (Gy)
1	14.59	(14.43, 14.74)
2	18.60	(18.16, 19.05)
3	21.05	(20.77, 21.33)
5	24.76	(24.39, 25.14)
6	26.05	(25.74, 26.36)
8	28.55	(27.95, 29.16)
10	30.28	(29.68, 30.91)
15	32.50	(31.74, 33.29)
20	35.56	(34.85, 36.29)

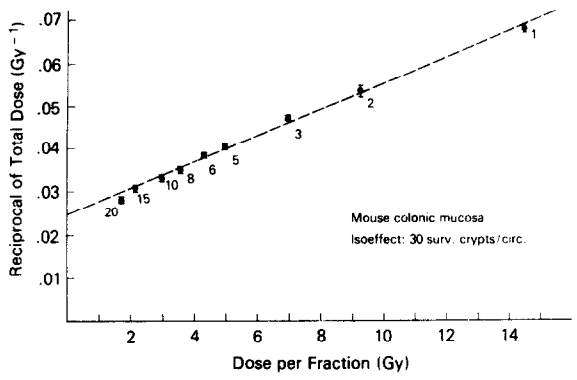

Fig. 1. Reciprocal total dose vs dose per fraction required to reach the isoeffect of 30 repopulated crypts per colonic circumference using multifraction regimens with 3-hr fractionation intervals. Numerals represent the number of fractions corresponding to each data point. The LQ model of cell survival predicts that these points will lie on a straight line $(\text{total dose})^{-1} = \alpha/E + (\beta/E)$ *(dose per fraction) provided that equal effect per fraction holds, where E is the log surviving fraction corresponding to the isoeffect. The 15-fraction data point is not significantly displaced from the regression line fitted to the data corresponding to 10 or fewer dose fractions, suggesting that the assumption of equal effect per fraction is valid for the regimens of 15 fractions. However, the* ED_{50} *for 20 fractions is somewhat larger than would be predicted by the remaining data, suggesting the probable occurrence of proliferation.*

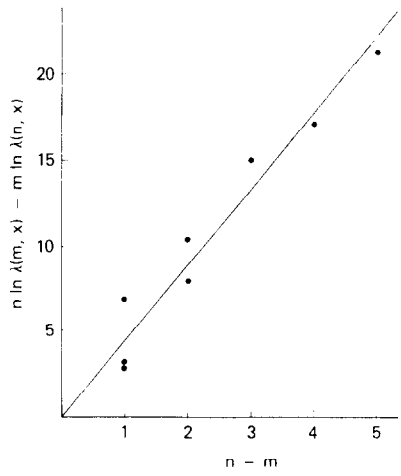

Fig. 2. Estimate of initial clonogen number. For each dose per fraction x given in regimens of m and n fractions separated by 3-hr intervals, the value $y = n \ln \lambda(m,x) - m \ln \lambda(n,x)$ *is computed from the cumulative cell survival levels* $\lambda(m,x)$ *and* $\lambda(n,x)$ *[cf. equation (2)]. The value y is plotted against (n−m) for each pair of common-dose regimens. Equal effect per fraction could not be rejected for these data since the intercept of the regression line did not differ significantly from zero. The slope of the line through the origin is an estimate of ln K, where K is the average initial per-crypt clonogen count.*

$y = n \ln \lambda(m,x) - m \ln \lambda(n,x)$ was computed from the average levels of per-crypt cell survival $\lambda(m,x)$ and $\lambda(n,x)$ that were observed after the total doses mx and nx. The resulting value is plotted against $n - m$ $(n > m)$ in Fig. 2. Since it was found that the hypothesis of equal effect per fraction could not

be rejected for these data, the slope of the regression line through the origin in Fig. 2 provides an estimate of $\ln K$ [cf. equation (4)]. The result is $K = 88$ with 95% confidence interval (55,140).

The estimate $K = 88$ of initial per-crypt clonogen count was used to construct a dose-survival curve for acute response in mouse colonic mucosa based on data from the single-dose experiments and the experiments with 3-hr fractionation intervals and fewer than 20 dose fractions. The fraction of surviving cells after dose x was determined by

$$\text{s.f.} = (\lambda(n,x)/88)^{1/n}$$

where $\lambda(n,x)$ is the average per-crypt cell survival after n successive doses of size x [cf. equation (2)]. The resulting dose-survival curve is shown in Fig. 3. Each of the following models of cell survival in semilogarithmic coordinates was fitted to the data:

$$\text{(LQ)} \quad \ln(\text{s.f.}) = -(\alpha x + \beta x^2)$$

$$\text{(TC)} \quad \ln(\text{s.f.}) = -ax + \ln(1 - (1 - e^{-bx})^n).$$

The parameters α, β, $a = ({}_1D_0)^{-1}$, $b = ({}_nD_0)^{-1}$, and n listed in Table 3 were obtained by a least-squares fit in which data were weighted by the reciprocal of the observed standard deviation resulting from replicate observations at the same dose.

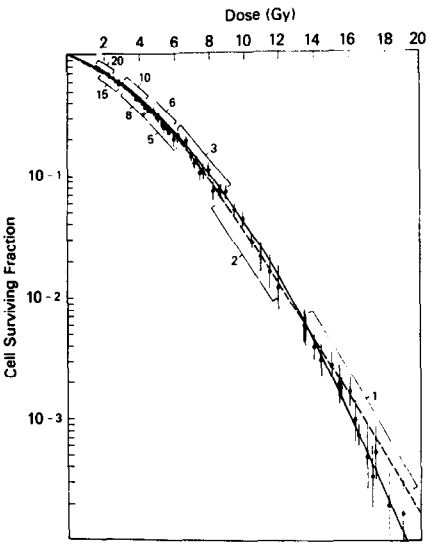

Fig. 3. Dose-survival curve for acute response in the mouse colonic mucosa. Each point represents the mean observed value from replicate observations (usually 10) at the same dose. Error bars represent ±1 standard deviation. Brackets indicate the contribution of data from each fraction number in the 3-hr multifraction experiments. The LQ (solid curve) and TC (dashed curve) parameters were estimated by least-squares fits to the data corresponding to fewer than 20 fractions.

DISCUSSION

An *in vivo* colony assay was used to measure the survival of clonogenic cells in the mouse colonic mucosa after irradiation. The average number λ of surviving cells per colonic crypt was estimated from the number of crypts that were repopulated within several days of completion of irradiation, with a correction based on the probability of multiple survivors in a regenerated crypt [cf. equation (2)].

A recently developed statistical method provided a test of the hypothesis of equal effect per fraction in the data obtained from regimens with 3-hr fractionation intervals. It was found that this hypothesis could not be rejected for the data from multifraction schemes of 10 or fewer fractions. A plot of reciprocal total dose vs dose per fraction for a fixed isoeffect suggested that this conclusion was also valid for the 15-fraction data, but that some proliferation probably occurred during the time required for the 20-fraction regimens.

Low-dose cell survival was determined from the results of the 3-hr multifraction experiments with fewer than 20 fractions, based on the assumption of equal effect per fraction. The surviving fraction at each dose was calculated by assuming that each colonic crypt contained an average of 88 clonogenic cells prior to irradiation. The estimate $K = 88$ was obtained using the statistical method described above [cf. equation (4) and Fig. 2]. The resulting surviving fraction data were used to obtain weighted least-squares fits of the para-

Table 3. Parameters of cell survival models for mouse colonic mucosa

Model	Parameters	Estimate and 95% confidence interval
LQ	α	0.147 (0.145, 0.149) Gy^{-1}
	β	0.0173 (0.0170, 0.0176) Gy^{-2})
TC	${}_1D_0$	5.43 (5.35, 5.52) Gy
	${}_nD_0$	2.81 (2.76, 2.86) Gy
	n	8.43 (7.90, 8.97)
	D_0	1.85 Gy
	D_q	3.94 Gy

meters of two standard survival models, the linear quadratic (LQ) and two-component (TC) models (cf. Table 3). As shown in Fig. 3, each of the two curves provides an adequate fit to the data for survival above 10^{-3}, although for lower survival levels the LQ fit appears to be slighly better.

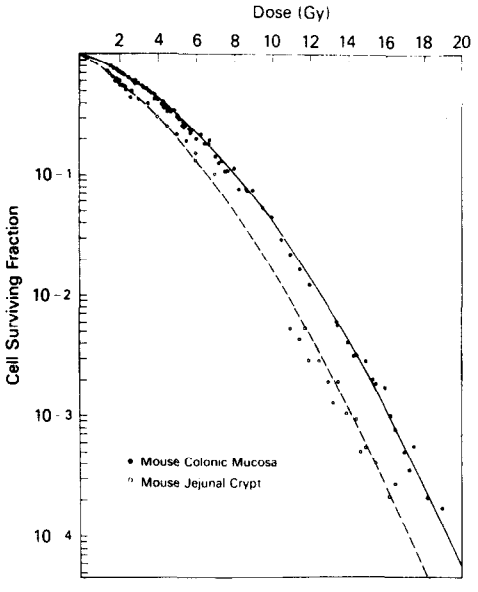

Fig. 4. Comparison of dose-survival curves for mouse colonic and jejunal mucosae. The solid and dashed curves represent the LQ models fitted to each of the data sets.

The (acute) survival characteristics of the mouse colonic mucosa are compared to those of the mouse jejunum [8] in Fig. 4. The survival curves for the two tissues indicate that the colonic mucosa is less radiosensitive than that of the jejunum. For a dose in the clinical dose range (2 Gy), survival of jejunal crypt cells is only 84% of that for cells of the colon. However, this result is dependent upon the accuracy of the initial per-crypt clonogen estimates for each of the tissues. The surviving fractions at 2 Gy would coincide if the estimate $K = 88$ were increased by about 20%.

The differences in cell survival predicted by the two curves of Fig. 4 is due primarily to the difference between their initial slopes [$\alpha = 0.147$ Gy^{-1} for colon vs $\alpha = 0.233 Gy^{-1}$, with 95% confidence interval (0.223,0.243 Gy^{-1}) for jejunum]. The coefficients of $(dose)^2$ from the LQ model are nearly identical for the two cell systems [$\beta = 0.0173$ Gy^{-2} for colon vs $\beta = 0.0177$ Gy^{-2}, with 95% confidence interval (0.0170,0.0176 Gy^{-2}) for jejunum]. A similar value of β [$\beta = 0.0172$ Gy^{-2}, 95% confidence interval (0.0162,0.0182 Gy^{-1})] has been found for the stem cells of the murine testis, based on data from an *in vivo* colony assay [5]. Rossi has presented theoretical arguments for the independence of β of radiation quality [9]. The β values quoted above for cells of the murine colon, jejunum and testis suggest that β might be independent of tissue type as well.

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