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Epilation in Rats After Single and Multifractionated γ-ray Exposure[1]

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一回および多分割ガンマ線照射に対する
ラットの毛髪の反応

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　ラット背部毛髪を使用して，一回および多分割ガンマ線照射した時の等効果線量を求めた．一回および多分割照射した時の線量効果関係を分析すると，次のことが暗示された．毛髪の telogen follicle 細胞の一回照射—効果曲線は，線量域500から2,300ラドの範囲では，片対数グラフ上にあらわして上に凸のカーブである．

　わずかではあるが，肉眼的にはつきりとわかる脱毛を指標にして，総照射期間（日）と必要な総線量との関係を両対数グラフ上にあらわすと，分割回数が2から8にふえるにつれて曲線の傾斜は0.08から0.30に増した．これは Ellis によつて提案された分割回数，総照射日数，そして等効果線量とを関係づける式は，仮に指数を変えても，少なくとも脱毛を指標とした障害にはあてはまらないことを示している．

Summary

The response of hair of rats to single and multifractionated gamma rays was studied using an arbitrary scale of epilation as an endpint. The dose response relationships for single and multifractionated treatments were analyzed. This analysis suggests that the single-dose survival curve for telogen follicle cells bends downwards continuously between doses of 500 and 2300 rads.

When the total doses for minimal definite epilation were plotted on a log-log scale as a function of overall time, the slope increased from 0.08 to 0.30, with an increase in number of fractions from 2 to 8. This suggests that Ellis' formula for a time-dose relationship can not be applied to the data in this paper.

Introduction

In a previous paper[4] it was shown, using the D_m- and D_h-concept, that single-dose survival curves of some normal cells exposed *in situ* to gamma rays had an initial exponential portion, followed by a

downward bending portion, which included one or two exponential segments. Although partial or complete epilation is not a dose-limiting factor in clinical radiotherapy, knowledge of single and multifraction dose survival curves for hair follicle cells is of basic radiobiological interest and may be useful for extrapolation to other tissues.

It is the purpose of this paper to demonstrate the response of hair follicle stem cells to single and multifractionated gamma rays and to demonstrate the characteristic of their single-dose survival curve.

Materials and Methods

Animals: Female rats (Sprague Dawley) aged 11 to 12 weeks, weighing 130 to 160 g, were used in all experiments. In the first several experiments, animals were anesthetized with sodium pentobarbital (Nembutal) given intraperitoneally (35 mg/kg body weight). However, about 50% of the animals died before being irradiated or less than one day after being injected and, as a result, chloral hydrate (400 mg/kg body weight) was substituted. These injections were given intraperitoneally 30 minutes before irradiation.

Irradiation: Each anesthetized animal was placed in a dorsal position in a well-ventilated Lucite box, the primary purpose being to irradiate a 2.5 cm length of dorsal spinal cord. The back of each animal was irradiated with gamma rays from a lateral aspect through a 2.5×1.0 cm window in a 5 cm thickness of lead. Gamma radiations were delivered at a dose rate of 153 rads per minute to the skin, using a ^{60}Co radiotherapy machine with a source to mid-rat distance of 80 cm. The field size was 25×25 cm at 80 cm from source. Coefficient of variation of the dose rate within this field was less than 5%. The dose rate was measured by lithium fluoride thermoluminescent dosimeters placed in a rat-shaped tissue equivalent phantom. The uncertainties in the skin dosimetry of a glancing lateral ^{60}Co field to a rat's (hairy) dorsum is appreciated. Efforts to quantitate the dose accurately have been prevented, but, it is worth noting that the skin and hair reactions appeared uniform throughout the fields. Regardless of the uncertainties in absolute dose due to skin sparing, fur thickness and varying angle of incidence of the beam, we consider that the relativity of the doses has been measured accurately.

Groups of at least 5 rats were used at each dose. The irradiation schedules were as follows:
Single dose

16 fractions in 15 days (1-day interval)

8 fractions in 7 days (1-day interval)

4 fractions in 3 days (1-day interval)

2 fractions in 1 day (1-day interval)

8 fractions in 14 days (2-day interval)

4 fractions in 15 days (5-day interval)

Table 1. Hair reactions

Score	Observations
0.5	Probable slight hair loss
1	Definite hair loss
2	Skin slightly visible through hair
3	Severe hair loss
4	Greater than 90% hair loss
5	All of hair lost with some necrosis of skin

2 fractions in 15 days (15-day interval)

Hair was not plucked artificially and, as a result, the telogen follicle cells were the target cells respon-sible for hair loss after irradiation. The hair reactions on the irradiated backs of the animals were scored with the naked eye every 2 weeks up to at least 17 weeks after the last irradiation, using the response scale shown in Table 1.

Results and Discussion

Loss of hair versus days after irradiation: Levels of epilation for each total dose group from each exposure schedule were averaged for each day and some of them are plotted as a function of time after the last exposure. Examples are shown in Fig. 1. The observed level of hair loss increased steadily with time up to about 9 to 13 weeks after the last exposure.
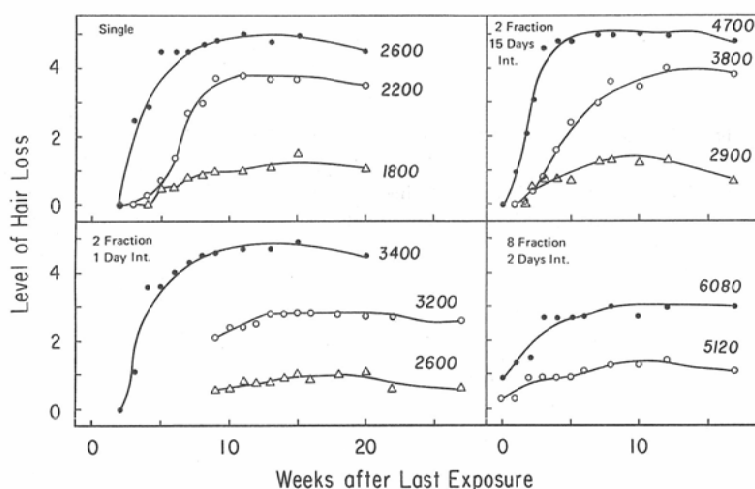

Fig. 1. Average level of hair loss following γ-ray exposures (single, 2 fraction-one day interval, 2 fraction-15 day interval and 8 fraction-2 day interval) versus weeks after the last exposure. Numbers shown in the figure are total dose (rads).

Dose response relationships: An average hair reaction in 6 successive weeks around the period at which the most severe reaction was observed was regarded as responses of each rat and was used to calculate the results in terms of hair reaction dose 50 (HRD 50) values. This value represents the dose necessary to elicit a certain average response in 50% of irradiated cases. For calculation of a particular HRD 50 value, the proportion of responders at each dose level was plotted as function of total dose and then a logit regression line was fitted to those data[5]. Figs. 2 and 3 show HRD_{50} values for various levels of epila-tion for single and multifractionated exposures. With an increase in number of fractions, not only the slope of the curve decreases, but the HRD 50 value for each level of hair reaction is shifted to a higher dose level.

Single-dose survival curve: It is difficult to determine directly the single-dose response curve of normal cells exposed *in situ*, especially to relatively low doses. On the basis of certain assumptions, however, the single-dose survival curve can be drawn using data from multifractionated exposures if the amount of
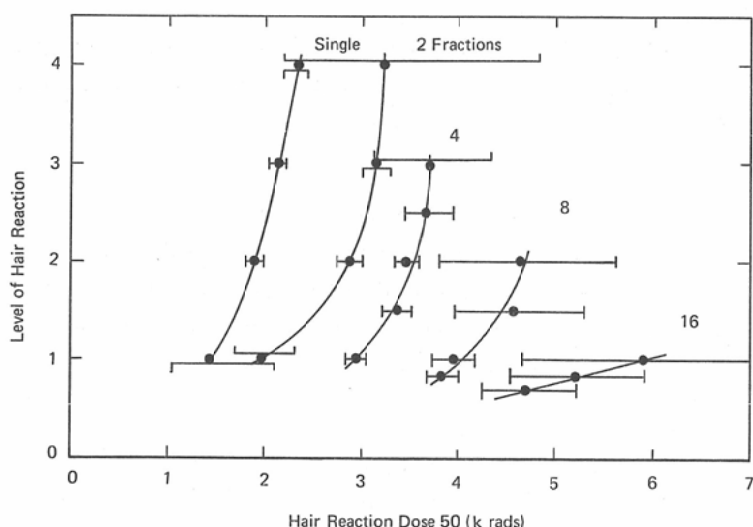
Fig. 2. Hair reaction dose 50 (HRD 50) value versus level of hair reaction used as an endpoint for rats exposed to a single dose, or 2, 4, 8 and 16 fractionated doses of γ-rays given at daily intervals; the horizontal lines represent 95% confidence limits around HRD values. Numbers shown on the figure refer to the number of fractions.
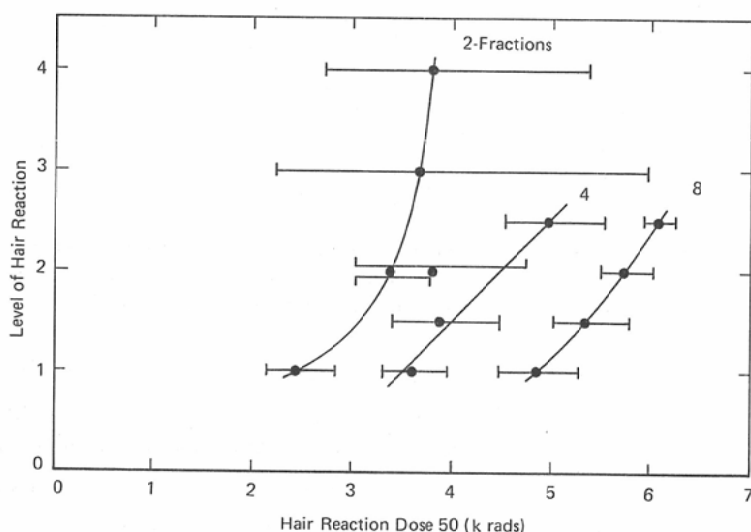

Fig. 3. Hair reaction dose 50 (HRD 50) value versus level of hair reaction used as an endpoint for rats exposed to 2, 4 and 8 fractionated doses of γ-rays in 14 or 15 days; the horizontal lines represent 95% confidence limits around HRD 50 value. Numbers shown in the figure refer to the number of fractions.

cell depletion corresponding to a given level of damage is determined[3]. Fowler and co-workers[2] presented another method of constructing a single-dose response curve, depending on the same basic idea, but providing less detail of the nature of the curve.

Our method is as follows:　Using data from multifractionated exposures, the relationship between a pair of points as close to each other as possible on a single-dose survival curve is established and a line passing through these two points is determined.　If many dose fractionation patterns and, correlated with this, a wide range of sizes of dose per fraction are used, then it is possible to show the characteristics of the single-dose survival curve of normal cells exposed *in situ* to a wide range of doses, including relatively low doses.　Details of this type of analysis have been published elsewhere[3].　Briefly, the following conditions should be assumed.

1.　Target cells do not multiply between the first and last exposure.

2.　The shape of the survival curve for each dose fraction is precisely the same as the shape of the single-exposure curve between zero and the dose used in each fraction.

3.　A given amount of damage scored by an arbitrary scale or a given number of colonies corresponds to a particular level of cell depletion in the target cell population (this level being responsible for observed damage), whatever irradiation schedule is used to obtain it.

That is, when the total dose in n fractions necessary to produce a given level of damage is D, the response to each dose fraction (or, in other words, a single dose D/n) is l/n of the given level of damage.

On these assumptions, a pair of points on a single-dose survival curve can be determined, using a pair of isoeffect doses from different multifraction exposure regimes.　The line passing through these two points on a single-dose survival curve can be characterized by two parameters: D_m and D_h (Fig. 4).
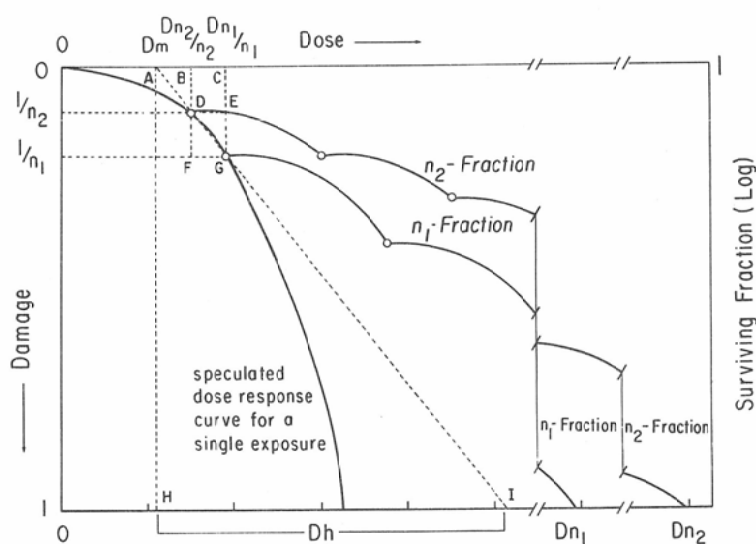

Fig. 4.　Model survival curves for multiple dose fractions. The bending curve is the single-dose survival curve. The upper solid line is the "effective" survival curve for n_2 equal fractions: the lower curve is for n_1 equal fractions. The total doses for an effect (denoted as 1) are Dn_2 given as n_2 fractions and Dn_1 given as n_1 fractions. D_m is the intercept with the zero-effect abscissa that results from extrapolating the segment D-G of the single-dose curve. D_h is a dose inversely related to the slope of the curve between D and G. (Reprinted with permission from British Journal of Radiology 49: 351–356 (1976).)

D_m and D_h, calculated by the following formulas, are the intercept dose with the abscissa at the level of no damage, and the inverse of the slope of the line passing through two (closely spaced) points, respectively.

$$D_m = (Dn_2 - Dn_1)/(n_2 - n_1)$$

$$D_h = (n_2 \sim Dn_1 - n_1 \sim Dn_2)/(n_2 - n_1)$$

where Dn_1 and Dn_2 are the total dose necessary to produce a given level of damage (isoeffect) in n_1- and n_2-equal exposures, respectively.

If the single-dose response curve is of so-called C-type, and if a pair of points from which D_m and D_h were calculated are on the exponential region of the curve, D_m is precisely equal to the D_q value and D_h is directly proportional to D_o.

If a single-dose survival curve is a simple exponential, the value of D_m and D_h will remain constant, D_m being zero. If a curve is downward bending, the value of D_h will increase and the value of D_m will decrease with increase in dose. Therefore, conversely, the relationship between pairs of doses and corresponding D_m- and D_h-values could show the nature of a single-dose survival curve.

The time interval between doses in this study was 24 hours, sufficient to permit complete recovery from sublethal damage. It is unknown whether or not this interval is optimum for the other assumptions that need to be satisfied for applying the D_m- and D_h-concept. However, if it can be assumed that the amount of recovery from sublethal damage is the biggest factor in the sparing effect of dose fractionation in the system studied, we can apply the D_m- and D_h-concept to the data. Fig. 5 shows the dose per fraction and the D_m- and D_h-value relationship for telogen follicle cells calculated from the data shown in Fig. 2. The value of D_m increases with increase in dose per fraction, except for the two linear portions; from 500 to 1600 rads per fraction where D_m remains around 300 to 500 rads, and from 1500 to 2300 rads
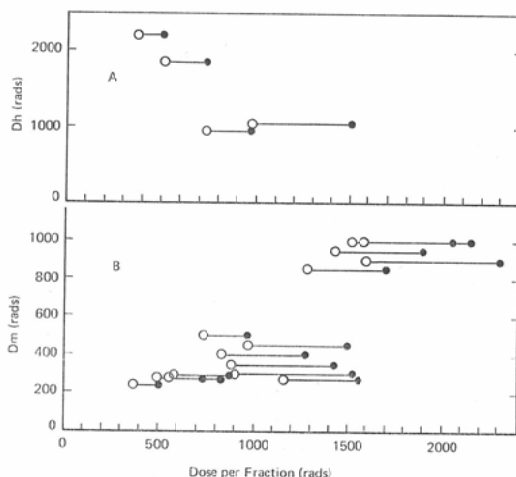

Fig. 5. The D_h (A) and D_m (B) vs. dose per fraction for telogen follicle cells of rats. D_h represents the dose necessary [to produce definite but minimum hair loss on a line passing through two points (shown as abscissa) of a single-dose response curve. Open and closed circles indicate, respectively, smaller and larger doses of a pair of isoeffect doses per fraction used to calculate D_m and D_h.

per fraction where it ranges around 900 to 1000 rads. D_h is the inverse of the slope of a limited part of the curve and is arbitrarily defined as the dose necessary to give a definite but minimum hair loss. The D_h decreases with an increase in dose per fraction (or a decrease in number of fractions).

These findings suggest that the single-dose survival curve of telogen follicle cells *in situ* may not be of the so-called C-type, which is common in the case of *in vitro* cells, nor simply a downward bending one, but may be more complex; that is, it is downward bending except for two exponential regions within the dose range tested. This shape for a single-dose survival curve of telogen follicle cells is similar to that of other normal cells *in situ*[4].

Time-number of fractions-dose relationship : A formula similar to the one proposed by Ellis[1] was applied to data on epilation to show the relationship between overall time, number of fractions and isoeffect dose. The curves for dose versus overall time for 2, 4 and 8 fractions were plotted on a log-log scale (Fig. 6A),
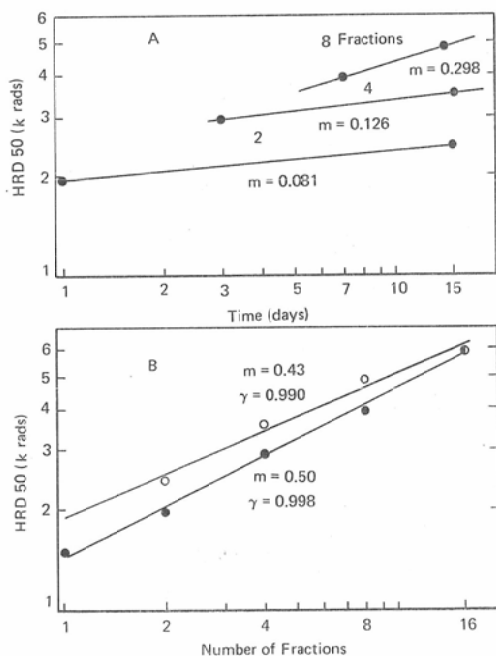

Fig. 6. Strandqvist type curves for HRD 50 (1) of rats exposed to gamma rays. (A) shows dose versus overall time for 2, 4 and 8 fractions, while (B) shows dose versus number of fractionated exposures in 14 or 15 days (○) and at one-day interval (●). Symbols m and γ refer to the slope of the curve and the correlation coefficient, respectively.

taking as an isoeffect the HRD 50 (1) (HRD 50 for grade 1 of epilation) from Figs. 2 and 3. The slopes of these lines, which give the exponent for T in the Ellis' formula, increased with increase in number of fractions. One slope would not fit to all the data. Similar results were obtained using data on HRD 50 (2) (not shown here).

The curve for total dose versus number of fractions for the isoeffect HRD 50 (1), for an overall time of 14 or 15 days, was plotted as a function of number of fractions (upper curve, Fig. 6B). The slope of

Table 2.　Epilation-fractionated exposure

Numbers of Fractions	Days	HRD 50 (1) (95% Conf. Limits) (rads)	ED*
1	—	1488 (1046—2116)	
2	1	1961 (1656—2322)	1460
4	3	2937 (2825—3053)	1500
8	7	3943 (3724—4175)	1408
16	15	5912 (4652—7515)	1485
2	15	2443 (2121—2815)	1488
4	15	3596 (3271—3955)	1630
8	14	4846 (4459—5266)	1644

*From formula $TD = ED \cdot N^{0.43} \cdot T^{0.07}$

the best fit curve was calculated to be 0.43.　As the slope for dose versus number of fractions for one day interval experiments was 0.50 (lower curve, Fig. 6B), the overall formula relating isoeffect total dose (TD), equivalent single dose (ED), number of fractions (N) and overall time in days (T) is $TD = ED \cdot N^{0.43} \cdot T^{0.07}$.

The exponent T determined in this way is almost the same as that calculated from data on 2 fraction experiments (Fig. 6A).　Therefore, the equivalent single doses have a wide range(Table 2). These results suggests that the Ellis' formula is not reasonable as an approximation for a time-dose relationship of this normal tissue.

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