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RAT SKIN CARCINOGENESIS AS A BASIS FOR ESTIMATING RISKS AT LOW DOSES AND DOSE RATES OF VARIOUS TYPES OF RADIATION

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INTRODUCTION

Estimates of the risks of leukemia and other cancers from exposure to relatively high doses and dose rates of ionizing radiation are available from epidemiological studies of various exposed populations, such as, the Japanese A-bomb survivors, patients irradiated for ankylosing spondylitis, etc. (1, 2). However, most occupational and environmental exposures occur at much lower doses and dose rates, and at the present time no generally accepted rationale exists for extrapolating risks from relatively high doses where data are available to low doses and dose rates where the data is either very poor or nonexistent (3). For making such extrapolations, not only must the dependence of tumor induction on dose be known, but also there must be information on possible effects of dose rate and age when exposures are extended over long periods of time.

Dose rate could significantly affect tumor induction because of the occurrence of recovery which tends to reduce the biological effectiveness of certain types of ionizing radiation (4). Quantitative effects of recovery on tumor induction have not been clearly established in epidemiological studies, although there is evidence from experiments with animals that low dose rates are less effective in producing tumors than high dose rates (5, 6). As irradiation controls improve, opportunities for epidemiological studies diminish and we must rely on experiments with animals for establishing the importance of dose rate, age, etc. on the induction of tumors. Ultimately the applicability of the animal data to the estimation of human risks will have to be established through an understanding of the general principles apply to different species.

An initial attempt to explain the role of recovery in tumor induction in rat skin has been made by postulating a two-stage model where one of the stages may be reversible (7). The dose-response function derived from the model consists of the sum of linear and quadratic terms and

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is in reasonable agreement with tumor induction data for electrons, protons and alpha particles. In the model, the dose rate effect on tumor induction depends on the recovery constant which can be measured experimentally by means of a split dose protocol.

The elements of the model are illustrated in Figure 1. Radiation may convert normal cells (designated S_1) to potentially neoplastic cells (designated S_3) by one of two routes, either a two step route with a reversible first step (designated S_2) or a route involving a single irreversible step. Each step is assumed to occur in proportion to dose in single cells, but the identity of the change and its site of occurrence within the cell need not be specified. If cells are converted to S_2 they may either revert back to S_1 or an equivalent state or be converted by further radiation action to S_3. The following differential equations describe these various transitions:

(1) $$\frac{d\bar{S}_2}{dt} = \bar{S}_1 K_{12} r - \bar{S}_2(\lambda + K_{23} r)$$

Type Within Solid Blue Lines

(2) $$\frac{d\bar{S}_3}{dt} = K_{23} r \bar{S}_2(t) + K_{13} r \bar{S}_1(t)$$

Where $\bar{S}$ represents the number of cells in the respective states, the K's are proportionality constants, λ is the recovery rate constant and r and t are the dose rate and exposure times respectively. Since the production of a few cancer cells is not expected to deplete significantly the relatively large population of normal cells, S_1 can be taken as constant. The exact solution for S_3 is somewhat complicated but can be simplified by considering certain limits. When the exposure time t is very short in comparison to the mean time cells spend in S_2, i.e., the exposure is 'acute' and $t << 1/(\lambda + K_{23} r)$ the solution is:

(3) $$S_{3a}(D)) = S(K_{13} D + \frac{K_{12} K_{23}}{2} D^2)$$

where the subscript 'a' indicates acute exposure. Equation 4 is a special case of the general form $S_{3a} = AD + BD^2$, i.e., a linear term plus a quadratic term (8).

Another limit exists when the dose rate is so low that $K_{23} r << \lambda$ and $t >> 1/\lambda$, i.e. cells enter S_2 much more slowly than they leave, and the exposure time is much longer than the reciprocal of λ. Within the above limits the solution is:

(4) $$S_{3p}(D)) = S(K_{13} D + \frac{K_{12} K_{23}}{\lambda} rD)$$

where 'p' indicates protracted exposure. In equation 4 S_{3p} is linear with total dose for a given dose rate and with dose rate for a given dose.

The model postulates an S_3 population which unfortunately cannot be detected directly but must be inferred by the presence of tumors. The relationship between tumor yield (y) and S_3 is assumed to be a simple proportionality, i.e., $y = CS_3$. Since C should be independent of dose, it must be assumed that events intervening between the formation of S_3 cells and their eventual expression as tumors are not influenced by radiation.

The quantitative expression of tumor yield may differ for different organs and types of tumors. Skin tumors tend to occur at a constant rate, I, after an initial tumor-free interval, and these rates were utilized as μ tune-independent measures of yield. Thus the measurable quantity, I, can be substituted for S_3 in equations 3 and 4 (9).

An initial question that needs to be answered is whether the general form of equation 3 is consistent with the experimental dose-response data, i.e. can the coefficients of the linear and quadratic terms in equation 3 be evaluated? This is best done by plotting the tumor response per unit dose versus dose, because in such a plot the data should be linear with a slope of B and an y axis intercept of A. Such data for the induction of tumors in rat skin with electrons, protons and alpha particles are shown in Figure 2. The data for electron and proton radiation indicate that if a linear term exists it must be very small and probably does not contribute more than about 10% to the total response. A measurable linear term does exist for alpha particles where the mean LET value is considerably higher than for electrons or protons.

The expected dependence of tumor response on dose rate is contained in equation 4 and can be expressed in terms of the response at high dose rates by defining a dose rate factor (DRF) as the ratio of dose (D_p) at low dose rate to dose (D_a) at high dose rate for the same tumor response. The DRF may be calculated by equating equations 3 and 4 and solving for D_a/D_p. The result is:

$$(5) \quad DRF = 1-K(1-2r/\lambda\ D_a)$$

Equation 5 specifies that the effect of dose rate, r, on tumor induction can be calculated for any given equivalent acute dose, D_a, provided values can be assigned to λ and K. The general form of K is the ratio of the effect produced solely by the two step mode to the total effect, i.e.

$$(6) \quad K = \frac{BD_a^2}{AD_a + BD_a^2}$$

In principle A and B would be determined from the dose-response curve, but as already noted A was too small to measure for electron radiation. If A were in fact zero, K would equal 1 and the expression for DRF would be:

(7) $$DRF = \frac{2r}{\lambda D_a}$$

Equation 7 indicates a progressively decreasing effectiveness with declining dose rate. For various mixtures of linear and quadratic terms the dose rate effect would occur in accordance with the relative magnitude of the quadratic term. If even a very tiny linear term exists, the DRF would approach a plateau of 1-K at low dose rates. The data in Figure 2 indicate that for electrons the linear term does not comprise more than 10% of the total response and could of course be much lower. On the basis of a model derived by Rossi and Kellerer from biphysical considerations, the linear term could be as low as 2% of the total response (10).

A value must be obtained for λ, the recovery constant, in order that DRF functions can be calculated numerically. Experiments were undertaken to measure λ for tumor induction on the basis of the rationale that after a given dose D_1 at high dose rate the persistence of S_2 cells would be indicated by the response to a second dose given at same later time, t. It can be calculated that S_2 cells ought to persist in accordance with the equation:

(8) $$S'_2(t) = S_2(0)e^{-\lambda t}$$

Equation 8 indicates that eventually the entire population of S_2 cells will be depleted but nevertheless D_1 itself will produce a response in accordance with equation 3.

For the measurement of λ, equation 8 must be expressed in terms of measurable quantities. A general expression for the amount of unrecovered effect, i.e. in the model the proportion of S_2 cells still remaining, can be derived as follows. If I represents tumor yield, the difference in response between split and single doses can be represented by $I(D_1,D_2,0)-I(D_1,D_2,t)$ where $D_1 + D_2 = D$ is the total dose given in two high dose rate fractions and t is the time between fractions. The zero in the first term indicates no time between exposures which is equivalent to a single dose of magnitude D. Since recovery is detectible by the difference in response between single and fractionated doses, it would be natural to express recovery quantitatively as the actual difference in response as a fraction of the maximum possible difference. Since the maximum difference in response would be expected if t were very long or effectively infinite, recovery (R) can be defined as follows:

(9) $$R = \frac{I(D,0) - I(D_1,D_2,t)}{I(D,0) - I(D_1,D_2,\alpha)}$$

Accordingly, the amount of effect not recovered is given by:

$$(10)\quad 1-R = \frac{I(D_1,D_2,t) - I(D_1,D_2,\alpha)}{I(D,O) - I(D_1,D_2,\alpha)}$$

which can be shown mathematically to be equivalent to $e^{-\lambda t}$ in equation 8. Hence equation 10 provides an experimental basis for the measurement of λ.

PROCEDURES AND MATERIALS USED

Male (CD strain) rats obtained from Charles River Co., Brookline, Mass. were housed two per cage and fed Purina Laboratory Chow (Ralston Purina, St. Louis, Mo.) and water ad libitum. The rats were irradiated at 28 days of age on a 2 x 5 cm area approximately centered on the dorsal skin surface. Three days prior to irradiation the hair was clipped and animals exhibiting hair regrowth within 7 days of irradiation were eliminated from the experiment in order to insure that all the animals were in the telogen (resting) phase of hair growth at the time of irradiation.

Irradiations were performed on the Van de Graaff accelerator at the Union Carbide Research Laboratory in Tuxedo, N.Y. The beam consisted of 0.7 Mev electrons at a current of 200 μA. The primary beam was far too intense for the direct exposure of the rats, and the dose rate was reduced by passing the beam through a 0.6 cm diameter orifice in a large (100 cm x 100 cm) lucite shield (0.6 cm in thickness) and by placing the rats as far as possible (130 cm) from the end of the beam pipe. The above configuration produced a radiation field with less than 10% dose variation sufficiently large to irradiate about 20 rats simultaneously.

Dose measurements were made with a 1.0 mm gap, parallel-plate ionization chamber. The electrons penetrated about 1.0 mm and results were expressed in terms of the dose at about 0.3 mm which has been found previously to correlate best with the tumor response (11). In the beam the dose rate was about 120 rads per min. The protocol of the experiment consisted of 9 single doses in order to establish the shape of the dose-response curve, and at three doses the exposures were split into two equal doses spaced at intervals of 15 min., 1 hr., 3.2 hrs. and 6.3 hrs. The irradiated area was outlined with a felt tipped pen to indicate the skin actually exposed to the radiation in order to insure proper alignment during reirradiation. About 5 min. prior to irradiation the rats were anesthetized with intraperitoneal injections of 30 mg/kg Nembutal (sodium pentobarbital) Abbott Laboratories, North Chicago, Ill.

Notations were made of the skin response every 6 or 8 weeks and photographs were made of each lesion when it was first observed and periodically thereafter. The tumor response in each observation interval was obtained as the average appearance rate of new tumors in the interval, and the cumulative response from the time of irradiation to the

midpoint of any later interval was the sum of appearance rates in preceding intervals. Specifically, if n were the number of new tumors in an interval, L the numbers of animals at the start of the interval and d the number of deaths in the interval, appearance rate was $n/(L-d/2)$. Sketches were made from the photographs in order that each tumor could be identified, assigned a time of occurrence, and examined histologically at the time of death. Only histologically-confirmed tumors were included in the analysis. The experiment was termined at either 52 weeks or 64 weeks and all rats surviving to these times were killed in order to obtain histological samples of the tumors.

RESULTS

For single doses the tumor appearance rates were generally constant after tumor-free intervals that ranged from 10 to 20 weeks. Mean rates and standard errors are shown in Figure 3 as a function of dose. The 'peaked' shape is typical of dose-response curves observed previously for rat skin and, as already indicated, the ascending limb is consistent with a dose-squared function.

Mean tumor appearance rates as a function of time between split doses are shown in Figure 4. For the lowest dose the data are somewhat variable, however, a general decline in tumor yield with time between doses was apparent. No residual effect of the first dose was detectible at 6 hours. Similarly for the intermediate dose, a declining trend with time between exposures is apparent. The increasing trend for the highest dose is also consistent with the occurrence of recovery in the sense that on the descending limb of the response curve a shift to lower effective doses would be expected to increase the yield.

In a separate experiment the effect of age at irradiation on tumor induction was evaluated by exposing rats ranging from newborn to 200 days of age to various doses of X-rays. It was found that newborn and weanling rats were about equally responsive while older rats tended to become progressively more resistant to the oncogenic effect of radiation until in 200 day old rats nearly 2.5 times as much dose was required in order to produce the same injury and tumor response as observed in the young rats.

DISCUSSION

For estimation of λ the data needs to be expressed as indicated in equation 10. Utilizing data from the smooth curve in Figure 1, the unrecovered suboncogenic effect as a function of time between doses for the two lower doses is shown in Figure 5. Possible lethal effects on the descending limb of the dose-response curve preclude the use of equation 10 for evaluation of λ at the highest dose. At the intermediate dose there was evidence of lethality and accordingly a correction was made by dividing the tumor yield

by the fraction of surviving hair follicles. The recovery halftimes at the lowest and intermediate doses were 1.4 hr. and 3.5 hr. respectively and, although this difference may indicate that recovery was dose-dependent, the calculation of dose rate effects was based on a mean λ of 0.4 $hr.^{-1}$.

With λ = 0.4 $hr.^{-1}$ the DRF functions for tumor induction are shown in Figure 6 for a total dose of 150 rads. If there were no linear term in the dose response curve, the curve labeled R = 1 would be expected. The curve labeled R = .90 would be expected if the linear term were 10% of the response and the curve labeled R = 0.98 would be expected if the linear term were 2% of the response. Only through additional experimentation can the appropriate curve be determined, but clearly in the dose rate range from 0.01 to 1.0 rads/hr. DRF values may range from 0.001 to 0.1 depending upon assumptions made about the nature of the dose-response curve.

For a given A and B values the value of R tends to decline as the dose declines and co-respondingly the DRF value rises, such that, as the dose approaches the dose of background radiation, i.e., in the range from 0.1 to 10 rads, the DRF becomes very nearly 1.0, and the effect of a dose given in minutes would be about equivalent to the effect of the same dose extended over a period of months or years.

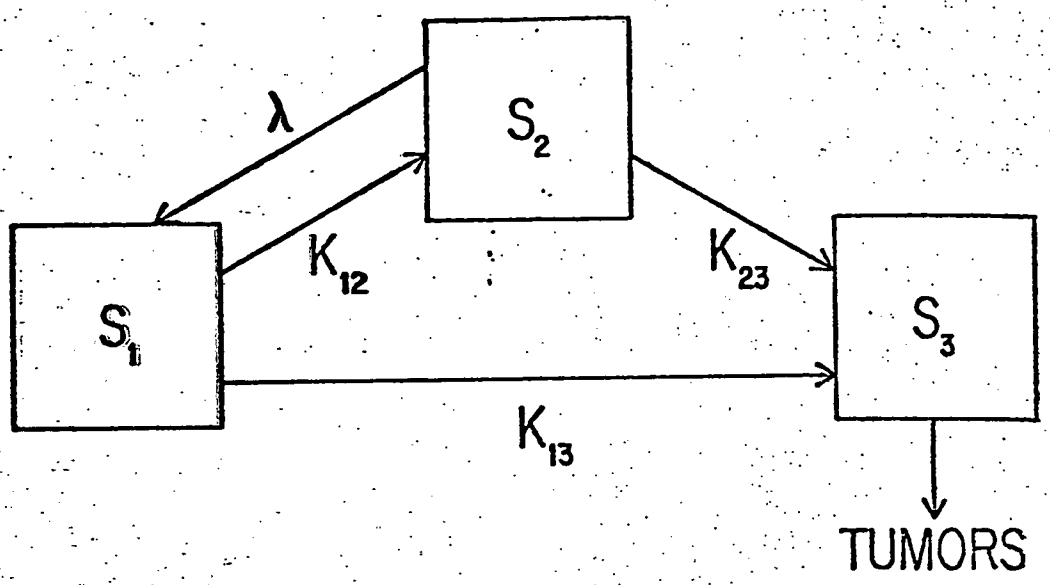
The general features of the DRF functions in Figure 6 may apply to other types of radiation provided that split-dose recovery can be demonstrated and λ values are comparable to values observed for electrons. So far the evidence suggests that for 24 hr. fractionation intervals, protons and X-rays exhibit considerable recovery although λ values are not available. Other types of radiation, such as, alpha particles, remain to be tested for recovery, although the possibility of a substantial linear term in the dose-response curve for alpha particles would tend to minimize the effect of recovery on dose rate.

The implication of these results and calculations is that dose rate could be an important determinant of the carcinogenic effect of radiation, especially in the intermediate ranges of dose and dose rate, such as might be encountered in certain occupational exposures. On the other hand, at very low doses the dose rate effect would be effectively abolished if the dose-response function contained even a very small linear term and at dose levels approaching background doses prudence would lead to the exclusion of dose rate effects on risk estimation.

SUMMARY

The recovery rate, age dependence and latent period for tumor induction in rat skin were measured for single and split doses of radiation, and the data were analyzed in terms of a general model in an attempt to estimate the expected tumor response for various types of radiation given at low

dose rates for long periods of time. The dorsal skin of male rats was exposed to electrons, X-rays or protons in either single or split doses for several doses and the tumor responses were compared during 80 weeks of observation. A two stage model incorporating a reversible or recoverable mode was developed and various parameters in the model, including recovery rate, dose-response coefficients and indices of age sensitivity, were evaluated experimentally. The measured parameters were then utilized to calculate expected tumor responses for exposure periods extending for duration of life. The calculations indicated that low dose rates could be markedly (1/100 to 1/1000) less effective in producing tumors than the same dose given in a short or acute exposure, although the magnitude of the reduction in effectiveness declines as the dose declines. The model fits the observed tumor response in rat skin reasonably well for acute exposures and the expectation of a greatly reduced effectiveness at low dose rates has sufficient support in the work of others to suggest that the model may be of value in estimating risks in humans especially in light of the similarities noted in tumor response for irradiation of human and rat skin.



S_1 = The initial state of unirradiated cells

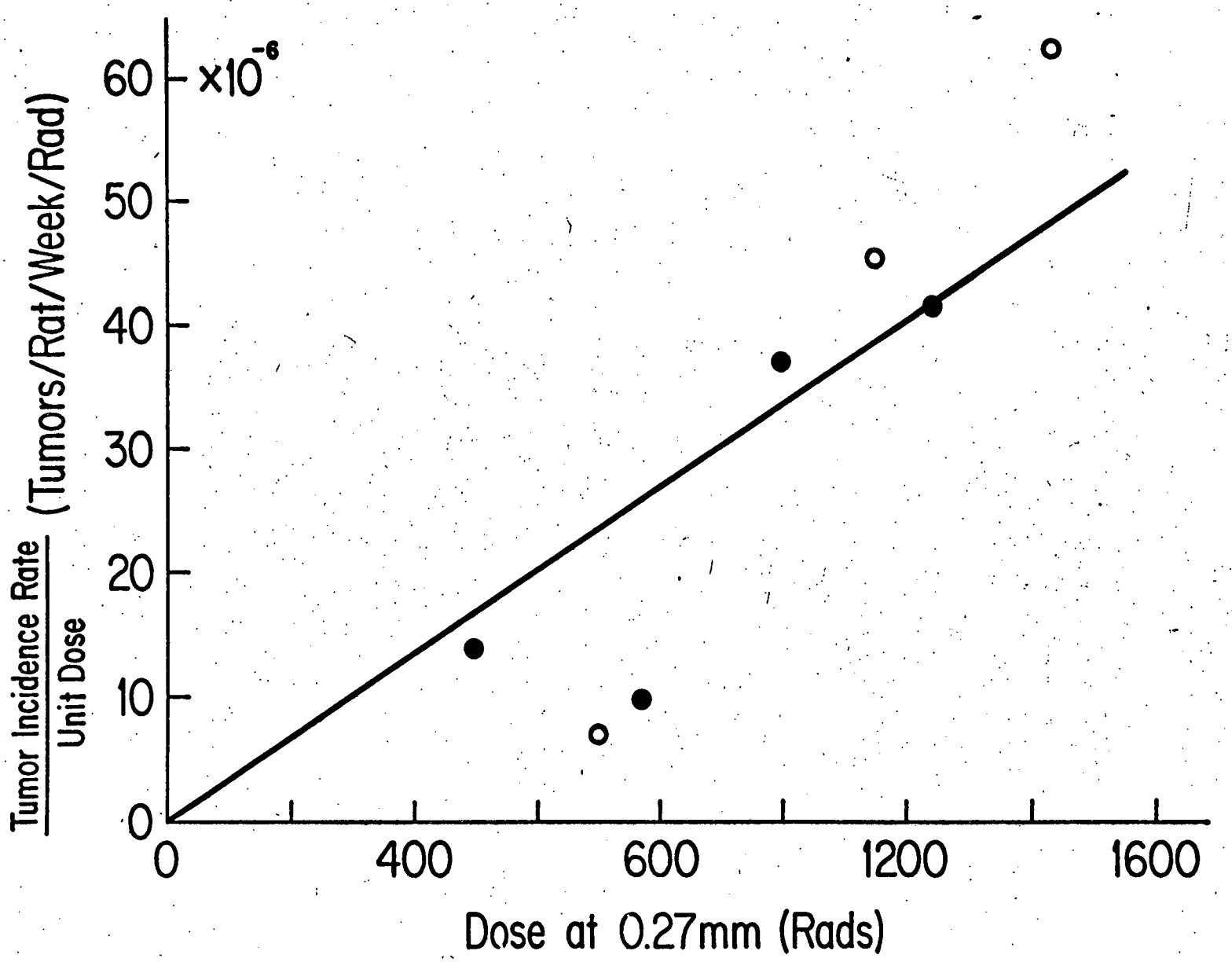
S_2 = The state of reversible, suboncogenically damaged cells

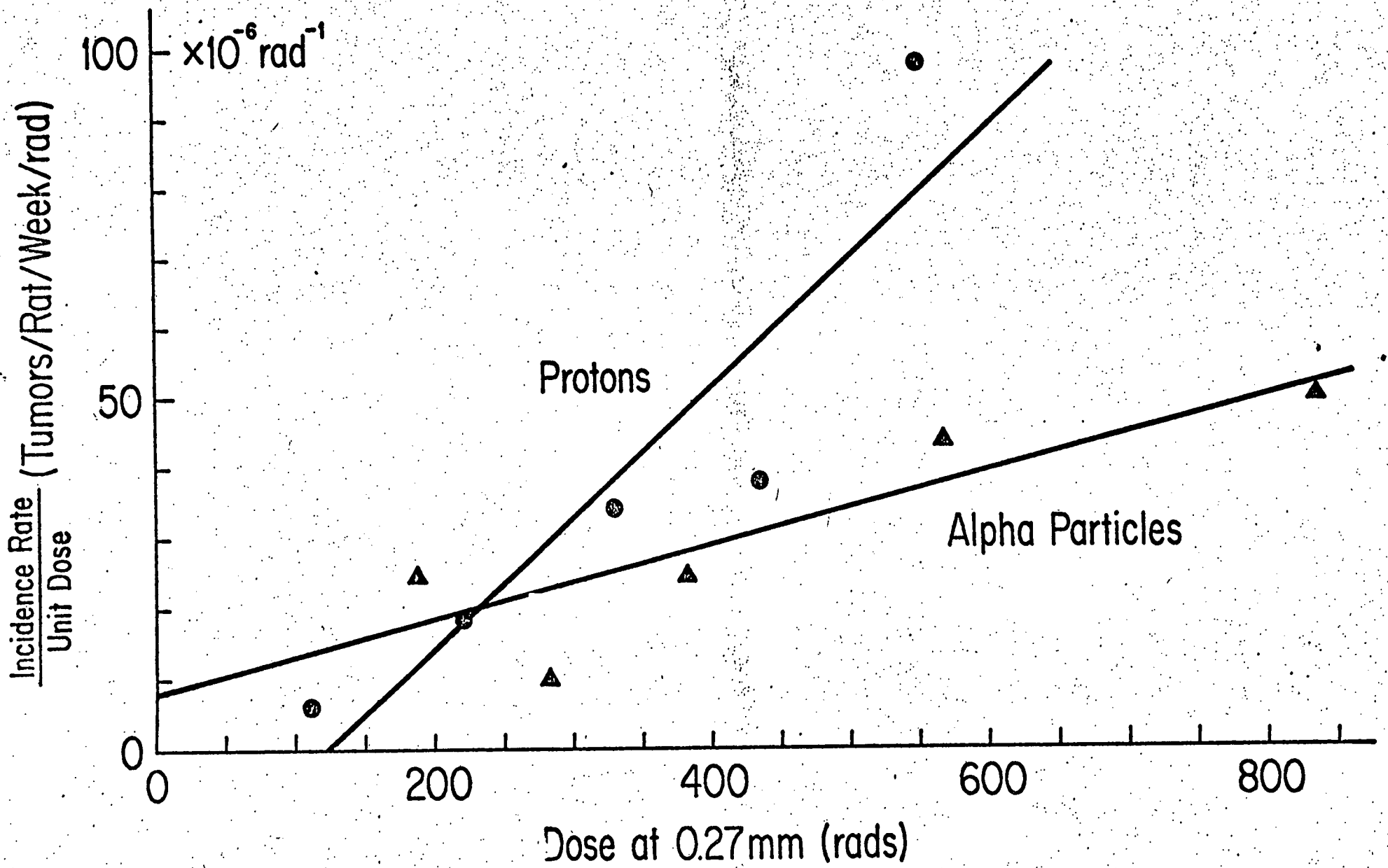
S_3 = The state of irreversibly damaged, potential tumor cells

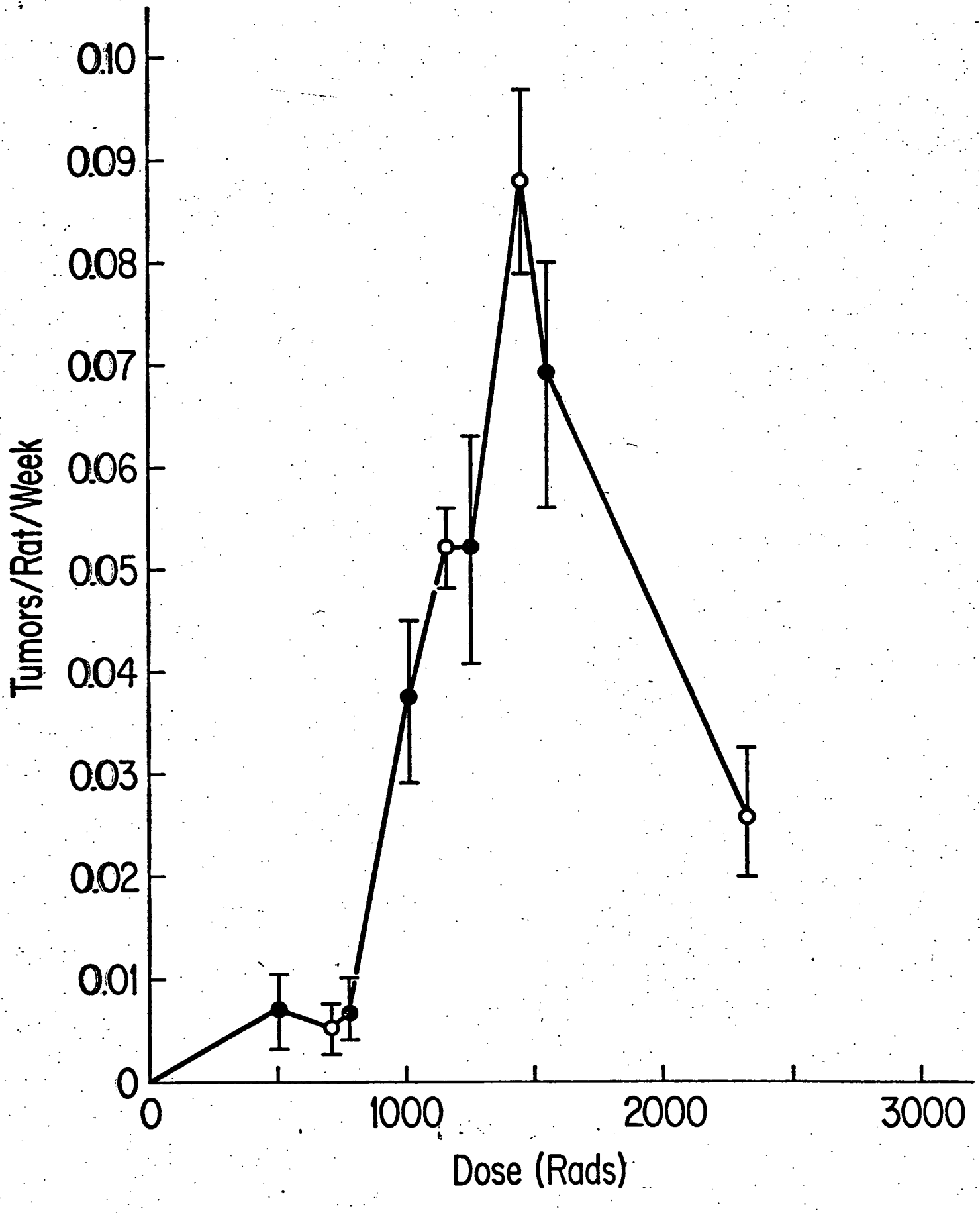
K_{ij} = The transition constant of cells from state i to j under the influence of radiation (units: $rads^{-1}$)

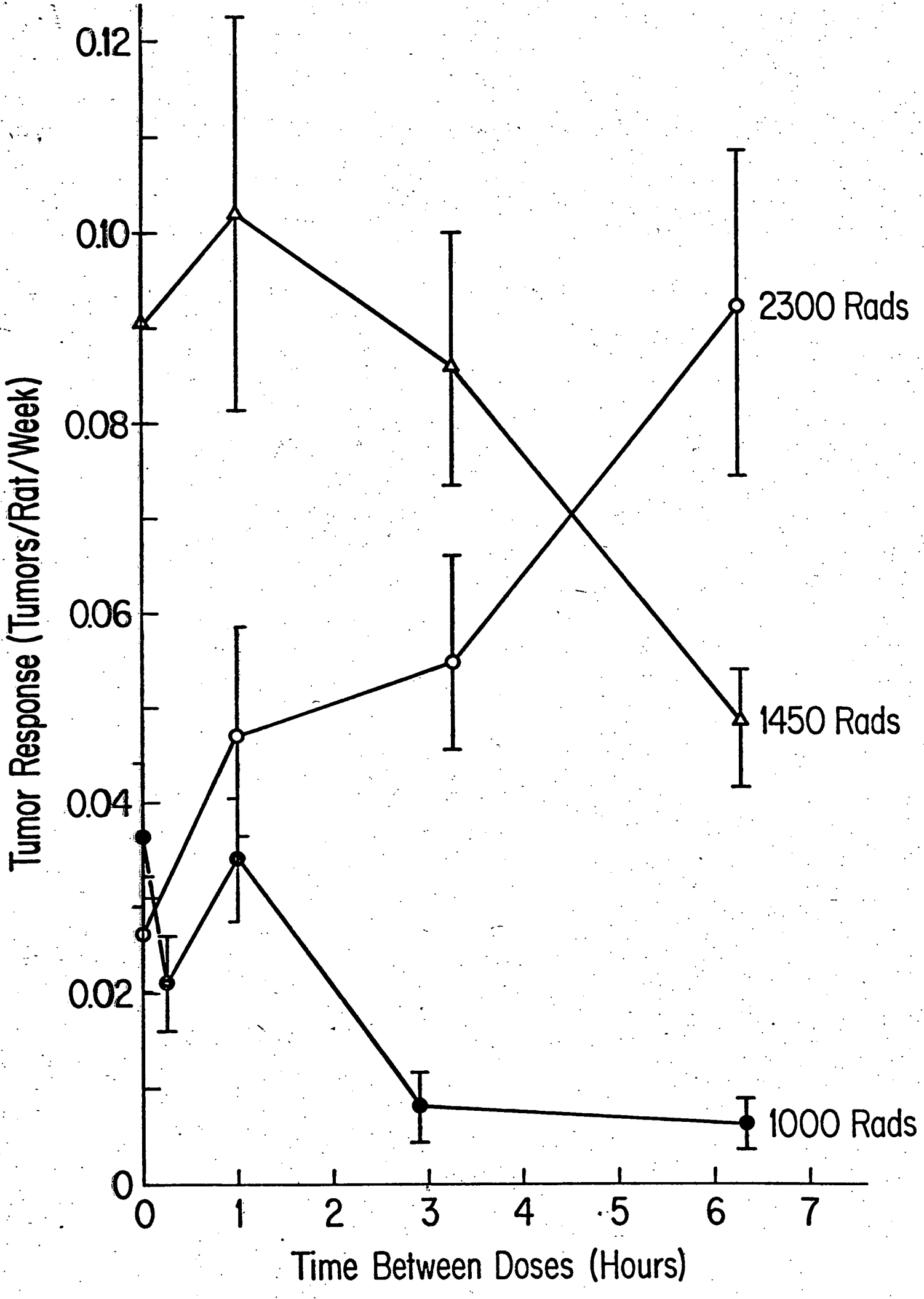
λ = The recovery rate constant of reversibly damaged cells (units: $hours^{-1}$)

Figure 1


Tumor Incidence Rate / Unit Dose (Tumors/Rat/Week/Rad)
×10⁻⁶
60
50
40
30
20
10
0
0
400
600
1200
1600
Dose at 0.27mm (Rads)


100
×10⁻⁶ rad⁻¹
50
0
Incidence Rate / Unit Dose (Tumors/Rat/Week/rad)
Protons
Alpha Particles
0
200
400
600
800
Dose at 0.27mm (rads)


0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Tumors/Rat/Week
0
1000
2000
3000
Dose (Rads)


0.12
0.10
0.08
0.06
0.04
0.02
0
Tumor Response (Tumors/Rat/Week)
2300 Rads
1450 Rads
1000 Rads
0
1
2
3
4
5
6
7
Time Between Doses (Hours)

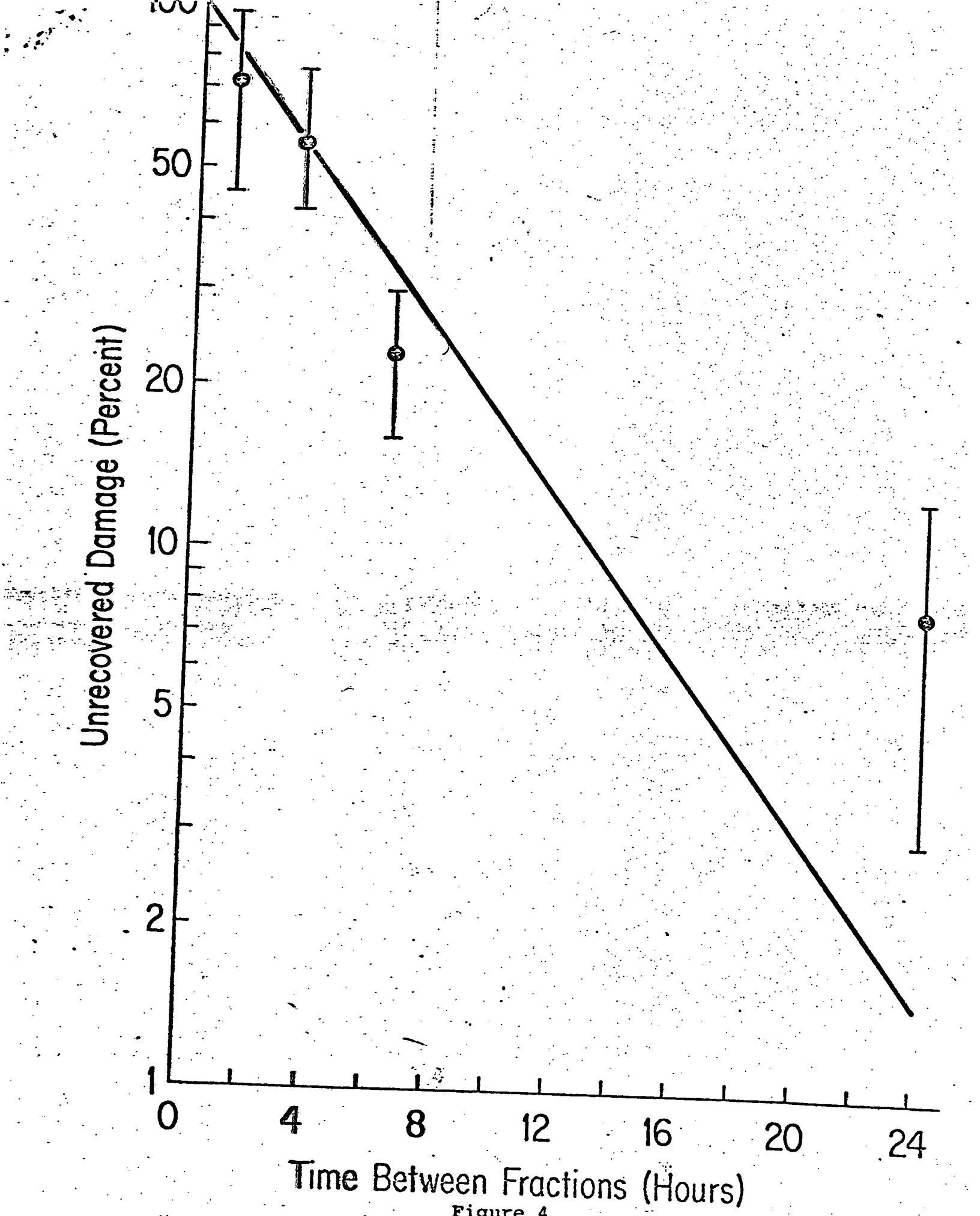

Unrecovered Damage (Percent)
100
50
20
10
5
2
1
0
4
8
12
16
20
24
Time Between Fractions (Hours)

Figure 4

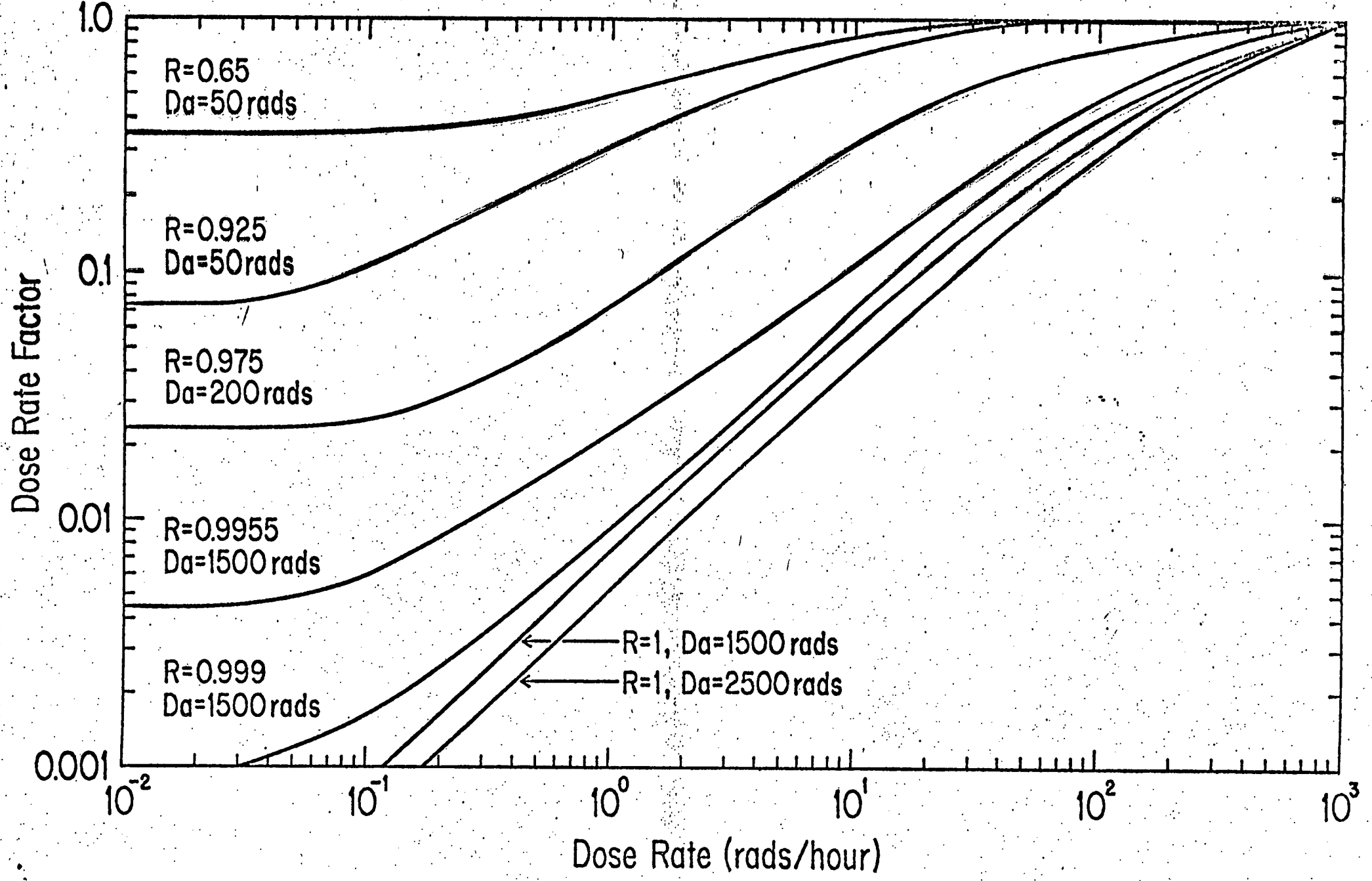

1.0
0.1
0.01
0.001
Dose Rate Factor
R=0.65
Da=50 rads
R=0.925
Da=50 rads
R=0.975
Da=200 rads
R=0.9955
Da=1500 rads
R=0.999
Da=1500 rads
R=1, Da=1500 rads
R=1, Da=2500 rads
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Dose Rate (rads/hour)

Figure 5