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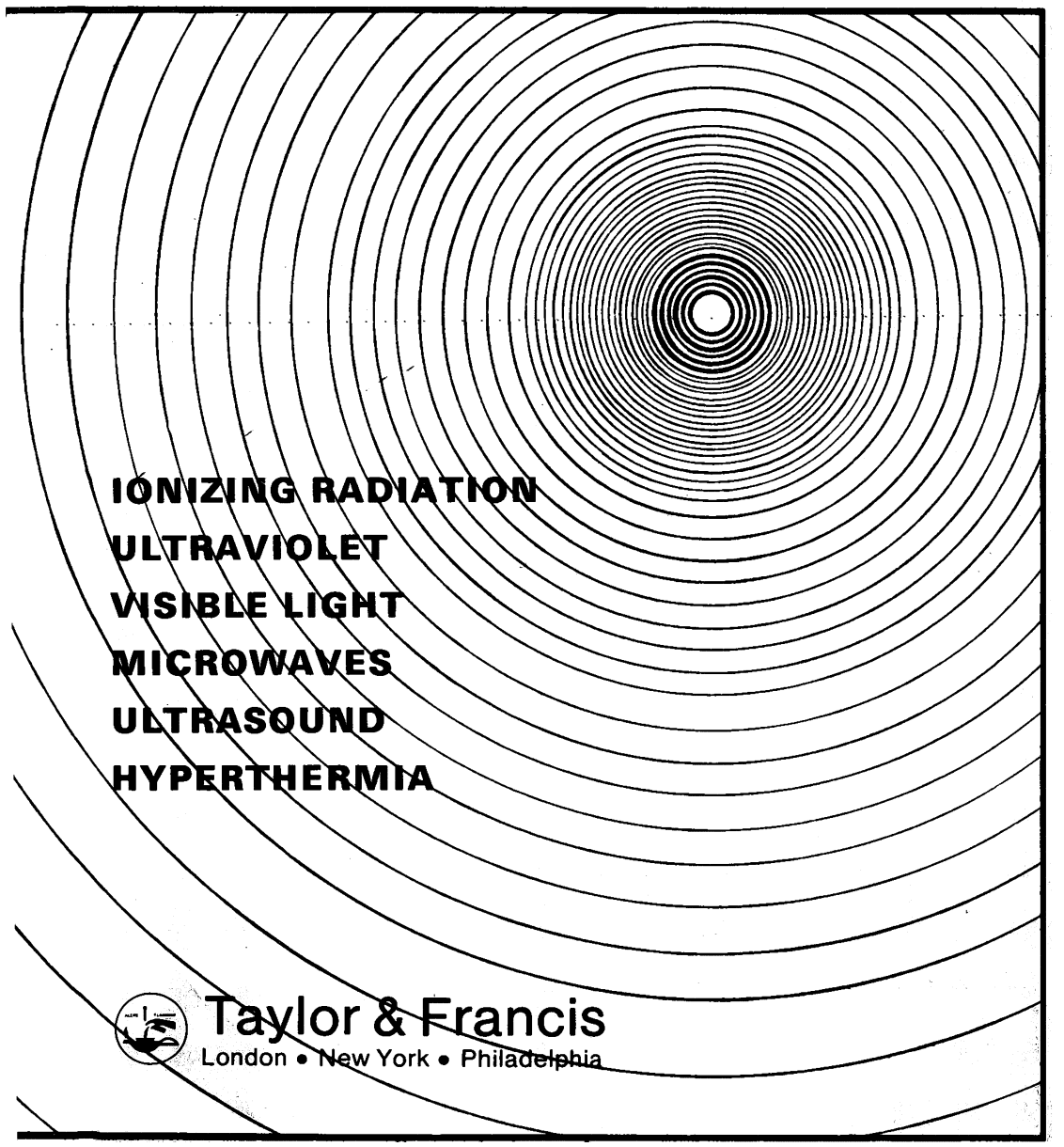

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Letter to the Editor

The dose rate dependence of oncogenic transformation by neutrons may be due to variation of response during the cell cycle

(*Received 18 April 1986*)

In recent communications, Barendsen (1985) and Elkind and Hill (1985) have discussed the observation by Hill *et al.* (1984) that reduction of the dose rate of 'fission' neutrons increases their effectiveness for transformation of C_3H 10T1/2 cells. This is a matter of substantial theoretical and pragmatic importance especially since the same phenomenon has also been repeatedly observed in *in vivo* carcinogenesis as pointed out before, e.g. by Sinclair (1982). Consequently there appears to be a major import to our understanding of radiation carcinogenesis and to radiation protection.

Barendsen's arguments based on microdosimetry appear to be sound. The reply by Elkind and Hill presents a possible formalism, but does not dispose of the basic problem. The term 'hit' has been employed in various ways, but if it is equated to a 'traversal' (in microdosimetric terms, an *event*), such traversals need not be assigned unit effect probability. The assumption would evidently not apply to this situation where, according to numbers quoted in the correspondence, at 10 mGy an event frequency (in the cell nucleus) of 0·1, results in a transformation frequency of 5×10^{-5}. As presented by Elkind and Hill, the theorem by Hall (1953) merely states, that if various factors (of which repair can be one and the amount of energy deposited certainly is another one) reduce the effectiveness of an event in whatever matrix it occurs by a factor, $k(<1)$, this still results in linear dependence of effect probability on event frequency. The question remains how k can depend on the absorbed dose rate of neutrons when the mean event frequency is said to be much less than one.

Event frequencies

The problem of event frequencies in the cell nucleus is crucial to the discussion, but has been treated somewhat inadequately and a more rigorous consideration is therefore required. Hill *et al.* (1984) state that at a dose of 10 mGy there is in the nucleus of a 10T1/2 cell a '90 per cent probability of single traversals (and therefore a 10 per cent probability of two or more traversals)'. Apparently the authors talk about probabilities among nuclei traversed at least once. Barendsen concludes from the statement: 'This implies that in a population of these cells irradiated with a dose of 10 mGy, in 90 per cent of the cells no energy is deposited at all, about 9 per cent are traversed by a single charged secondary particle and approximately 1 per cent of the cells are traversed by two or more particles'. From this it would follow that the event frequency in the nucleus of the cell is $\phi = 11/\text{Gy}$, i.e. that there is roughly 1 event per 100 mGy.

However, the original statement by Hill *et al.* implies an event frequency $\phi = 21/\text{Gy}$, as can readily be shown on the basis of Poissonian statistics. Furthermore, the geometry of the 10T1/2 cells and microdosimetric data suggest an even higher event frequency.

One of us (A.M.K.) has examined samples of stained 10T1/2 cells from colony tests at moderate cell densities. Averaging sizes over the variations that must evidently occur in an exponential population one deduces a mean projected nuclear

area which corresponds roughly to the area of a disc with a 17 μm diameter, or about 230 μm^2. If the nucleus is rather flat, its total surface will be slightly more than twice the projected area. Thus these measurements indicate—even without adjustment for shrinkage during fixation—a surface of the nucleus of roughly 500 μm^2. Geard and Harding (1981) report a mean nuclear cross-section of non-fixed cells that is about twice as large 2 days after plating, while it approaches the value of 250 μm^2 after several additional days of incubation. The larger value would seem to apply, because Hill, Han, and Elkind performed the irradiations 2–3 days after plating; however, 750 μm^2 will be chosen as a (possibly conservative) value of the nuclear surface.

For isotropic radiation each surface element is traversed by the same mean number of particles, and the number of particles entering a site is, therefore, proportional to its surface. However, because of the limited range of neutron recoils the event frequency is larger by a factor $f=(1+\bar{l}/\bar{r})$, where $\bar{l}$ is the mean chord length of the site and $\bar{r}$ is the mean range of the recoils (see generalized Cauchy theorem (Kellerer 1971)). Since f depends merely on the mean chord length of the site, one obtains the event frequency in a site as the event frequency in a sphere of equal mean chord length times the ratio of the surfaces of the site and the sphere. If the nucleus of the 10T1/2 cells is taken to be a slab of height 2 μm, the mean chord length is 4 μm, and the corresponding sphere has a 6 μm diameter. In a publication by Bateman *et al.* (1968) event frequencies as a function of sphere diameter, d, are given for neutron energies between 0·43 and 1·8 MeV which encompasses most of the spectrum at Janus. At $d=6$ μm all the values happen to be close to $\phi=6{\cdot}3$/Gy. The estimated event frequency in the cell nucleus is therefore about $750\phi(36\pi)=42$/Gy. If the nuclear thickness is somewhat larger, the event frequency is correspondingly greater. The fact that the recoils are primarily ejected normal to the projected area of the nucleus increases the number of particles incident per unit surface. Taking this fact into account one estimates an event frequency of roughly $\phi=60$/Gy.

It may be noted that the assumption that the gross sensitive volume is restricted to the nuclear volume, while reasonable, may not be correct and that even larger frequencies would be involved if it were comparable to the cell volume.

Possibility of a dose rate dependence at low event frequencies

Barendsen surmises that the observed dose dependences may be inconsistent with any autonomous action on individual cell nuclei at the event frequencies (10/Gy) which had been assumed. The argument may appear less relevant if the event frequencies are substantially larger. However, the essence of the discussion is fundamental to radiobiology, to radiation protection and to the application of microdosimetric data. It is therefore desirable to examine first whether one must, on the basis of general principles, discount the possibility of a dose rate dependence at event frequencies substantially less than 1.

To separate basic physical and mathematical arguments from assumptions concerning radiobiological mechanisms one can rephrase the problem posed by Barendsen in a somewhat formalized way.

If a dose rate dependence at event frequencies substantially below unity is impossible, it should also be impossible to conceive a detector that has a sensitive volume equivalent in size to the cell nucleus and a response probability nearly proportional to dose with pronounced dose rate dependence. Conversely, if such a detector is conceivable, a dose rate dependence cannot be excluded in principle. It may merely be implausible in view of radiobiological arguments.

Assume an event frequency of $\phi = 10/\text{Gy}$ in the sensitive volume of a detector. Assume further that a hypothetical detector responds with probability a to an event, but with probability $(a+b)$ to an event if it is preceded by another event with temporal separation not less than ΔT and not more than $\Delta T + T$. Evidently the postulate of a subsequent, rather than a preceding, event would be equivalent. But the allusion to the induction of an error-prone repair system after a delay ΔT and for a duration T may be illustrative.

At high dose rates, with exposure times less than ΔT, there can be no enhancing events. The response rate is then:

$$P(D) = a\phi D \tag{1}$$

At low dose rates, on the other hand, with exposure times in excess of $\Delta T + T$ an event can be preceded by a number of enhancing events which is proportional to the dose rate and the duration T. The response rate is then higher, but again nearly proportional to dose. The exact dependence for a constant dose rate $\dot{D}$ and for exposure times in excess of $\Delta T + T$ is cited here without derivation:

$$P(D) = k(D - \delta) \tag{2}$$

with

$$k = (a + pb)\phi \quad \text{and} \quad p = 1 - \exp(-\phi \dot{D} T)$$

and

$$\delta = \dot{D}[\Delta T + T(1 - 1/p - 1/\ln(1-p))]/(1 + a/pb), \quad \text{i.e. } \delta < \dot{D}(\Delta T + T/2)$$

With $\Delta T = 5$ min and $a = 6 \times 10^{-5}$ and for durations of exposure less than 5 min one obtains the slope 6×10^{-4}/Gy which agrees with the data by Hill *et al.* for high dose rates. The values $b = 2 \times 10^{-3}$ and $T = 30$ min result for the low dose rate 0·86 mGy/min in the slope $k = 5{\cdot}3 \times 10^{-3}$/Gy which also agrees with the value given by Hill *et al.* The term δ equals 15 mGy, and the deviation from dose proportionality could therefore be ascertained only with larger samples and at smaller doses than employed in the experiments of Hill *et al.*

The observed dose rate dependence can be obtained even at much lower event frequencies if a larger coefficient b is assumed. Although this is not demonstrated here, the assumed parameters are in general agreement also with the explicit dose rate dependence determined by Hill *et al.* (1984) for a dose of 210 mGy.

From the agreement of the response, $P(D)$, with the experimental data of Hill *et al.*, it follows that the results of these experiments cannot be rejected as inconsistent with basic principles of microdosimetry and the Poisson statistics of energy deposition. It is a different matter, whether the postulate of a greatly enhanced response for a particular temporal correlation of two events may have biological reality. Barendsen addressed in his argumentation the possibility of an enhancing event preceding the second event, with a constraint on the maximum, but not on the minimum, temporal separation. He observed correctly that this could not lead to a linear dependence at low doses and dose rates. The reaction characteristic which has here been considered is hypothetical; it shows merely that the 'paradoxical' dose rate dependence is possible in principle. The likelihood of a more plausible cause of the dose rate dependence at low doses makes it desirable to investigate another explanation.

Implications of a brief period of high sensitivity

In the earlier communications it is acknowledged that at sufficiently low doses the transformation yield must be independent of dose rate. However, Barendsen's statement that this implies a change of the low dose rate curve seems inadequately justified, and it is *a priori* equally possible that the high dose rate curve joins the low dose rate curve below about 10 mGy. This is indicated in figure 1 by curves which will be explained subsequently. In acute irradiation such dependencies indicate a small but highly sensitive fraction of the population.

A possible explanation is that during the cell cycle there is a brief period of much higher sensitivity to transformation (or that there might be several such periods) and that protraction of irradiation increases the number of cells irradiated during this phase. If the mean number of events corresponding to a given absorbed dose is larger than that required for transformation, a high dose rate results in a smaller number of cells receiving an excessive amount of energy, lessening the effectiveness. The precise numerical aspects of this hypothesis must be based on data that are, at present, unavailable. However, there are some semi-quantitative arguments that support it.

In a first approximation it may be assumed that the cells can be transformed with a much higher frequency during a brief period, τ, of the cell cycle. The fraction of these cells is then roughly equal to τ/S, where S is the duration of the cell cycle. Depending on the position of the sensitive phase in the cycle the value may be somewhat different in an exponential population; however, such deviations are immaterial to the subsequent considerations and the value τ/S can therefore be used.

In view of the small absorbed doses involved only single event action will be considered. While multi-event action seems unlikely in the case of neutrons it may well be important in the case of low-LET radiation. The additional complexity should however not affect the basic mechanism proposed here. It should be noted

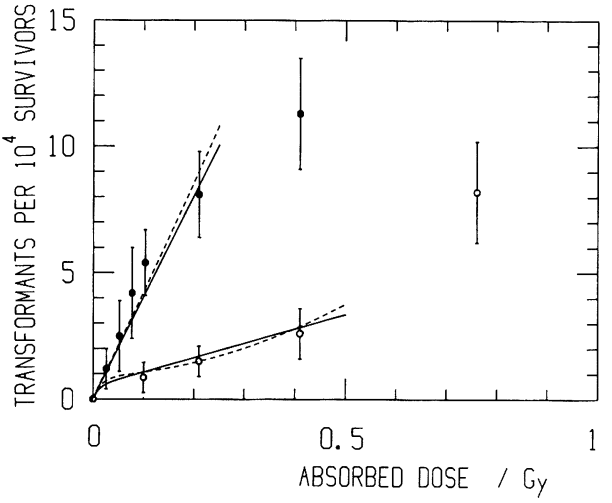

Figure 1. Transformation frequencies of C3H 10T1/2 cells exposed to fission neutrons at 0·86 mGy/min (●) and 103 or 380 mGy/min (○) according to data by Hill *et al.* (1984). The solid and the dotted lines are fits to the data which indicate that the high dose-rate curve can approach the low dose-rate curve at low doses. The curves are obtained as explained in detail by figure 3.

that following the physical process of energy deposition the overt expression of transformation is likely to be influenced by various factors, as has been pointed out by numerous authors. This point will be taken up below and the term 'alteration' will be employed here for what is sometimes termed 'pre-transformation' to specify the initial injury of the cell.

If at an absorbed dose D the mean number of events effecting the alteration of these cells is λD, the fraction of the population altered (when the sensitivity outside the critical phase is neglected) is:

$$A_0 = (1 - \exp(-\lambda D))\tau/S \tag{3}$$

when the exposure is instantaneous.

When the absorbed dose is administered at a rate $\dot{D} = D/t$ (where t is the exposure period) and if cell doubling by mitosis is neglected, the fraction of cells irradiated during the sensitive phase is increased to $(t+\tau)/S$, but the average† absorbed dose during the sensitive phase is reduced to $D\tau/(t+\tau)$ leading to:

$$A = (1 - \exp(-\lambda D\tau/(t+\tau))(t+\tau)/S. \tag{4}$$

For constant dose rate, $\dot{D}$, the dose dependence is:

$$A = (1 - \exp(-\lambda D/(D/\tau\dot{D}+1))(D/\tau\dot{D}+1)\tau/S. \tag{5}$$

The initial slope is equal to $\lambda\tau/S$ for eqns. (3) and (5). According to eqn. (5) the slope decreases by the factor $(1 - \exp(-\lambda\tau\dot{D}))$ at larger doses. With eqn. (3) a plateau is attained at τ/S; the value λ equals, therefore, the initial slope divided by the magnitude of A_0 at the plateau. The solutions are represented in figure 2.

In these relations no account is taken of those transformations which are induced outside the sensitive phase, and which may contribute the bulk of the observed rate at high doses, where the data by Hill *et al.* indicate an equal rate of increase of

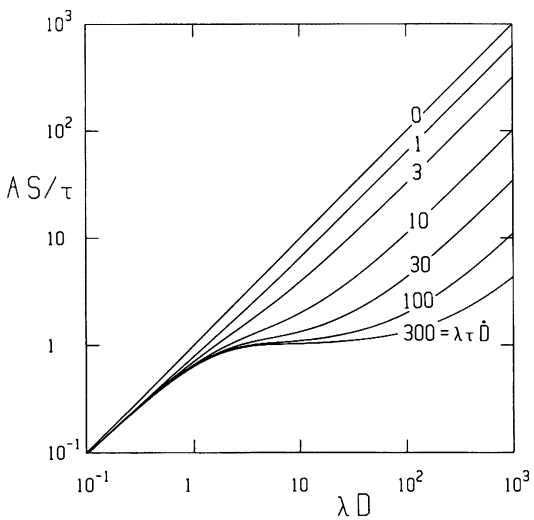

Figure 2. The alteration frequency as a function of dose at different does rates according to eqn. (5).

†A more detailed analysis shows that the average dose represents an adequate approximation.

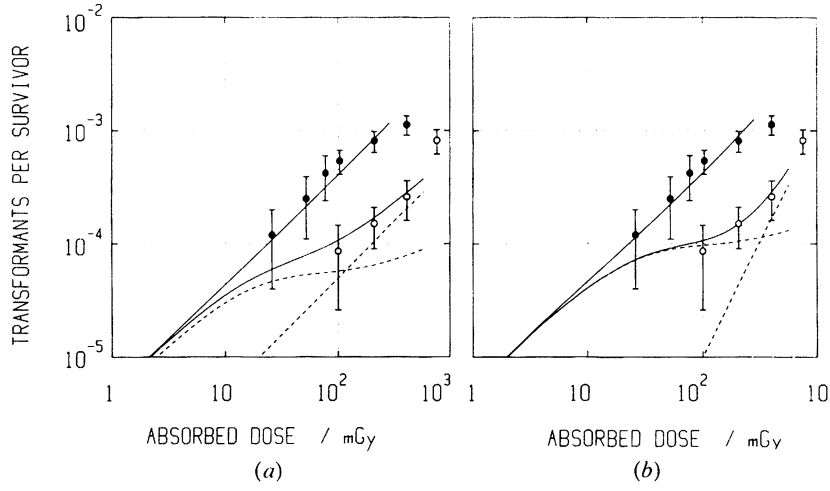

Figure 3. The curves fitted to the low dose data of Hill *et al.* (1984) under the assumption of a brief phase of enhanced sensitivity to transformation. (*a*) The parameters $\tau = 7$ min, $\lambda = 0{\cdot}09$/mGy, and $C = 7{\cdot}2 \times 10^{-6}$/min are employed and a linear dose dependence outside the sensitive phase with the dose coefficient 5×10^{-7}/mGy is assumed. (*b*) The parameters are $\tau = 12$ min, $\lambda = 0{\cdot}06$/mGy, and $C = 7{\cdot}5 \times 10^{-6}$/min, and a quadratic dependence on dose is assumed outside the sensitive phase with the coefficient 10^{-9}/mGy^2. The high dose rate curves correspond to $\dot{D} = 103$ mGy/min. The actual dose rate, 380 mGy/min, applied at $D = 410$ mGy corresponds to nearly the same transformation frequency ($1{\cdot}4 \times 10^{-4}$, instead of $1{\cdot}5 \times 10^{-4}$).

transformation rate at high and low dose rates. Fitting the data one must therefore invoke the additional component which is dose rate independent and which may be assumed to be either linear or quadratic in dose. Figures 3 (*a*) and (*b*) give possible fits to the low dose data according to eqn. (5) with the addition of a term which is linear (*a*) or quadratic in dose (*b*). A factor $C = \varepsilon/S$ instead of $1/S$ is used with eqn. (5) to account for the quotient, ε, between the observed transformation frequency and the alteration frequency.

The initial slope of the fitted curve in figure 3 (*a*) is $4{\cdot}5 \times 10^{-3}$/Gy and the plateau value (broken curve) is 5×10^{-5}. The excess of the implied value $\lambda = 90$/Gy over the estimated event frequency of 60/Gy in the nucleus of the 10T1/2 cells lies within the uncertainty of the data and the approximations of the model.

The value of τ can be estimated on the basis of the assumption that the quotient, ε, between the observed transformation frequency and the alteration frequency is the same for the sensitive cells as for the entire population. The latter might be taken to be 10^{-2} since this is near the maximum transformation rate observed among survivors at very high doses. With a plateau near 5×10^{-5}, τ/S can be estimated to be 5×10^{-3} which, with a cell cycle time of 24 h, indicates a sensitive period of 7 min. This value has been used in the fit of figure 3 (*a*).

Figure 3 (*b*) gives a fit obtained with the assumption of a transformation rate of the cells outside the sensitive phase that is proportional to the square of the dose (broken straight line). With this fit a somewhat larger plateau value results (broken curve), and one deduces the smaller value $\lambda = 60$/Gy which happens to coincide with the estimated event frequency in the cell nucleus. The fit requires also the somewhat larger value $\tau = 12$ min of the duration of the sensitive phase. Figure 1 gives the same fits as figure 3 in linear representation.

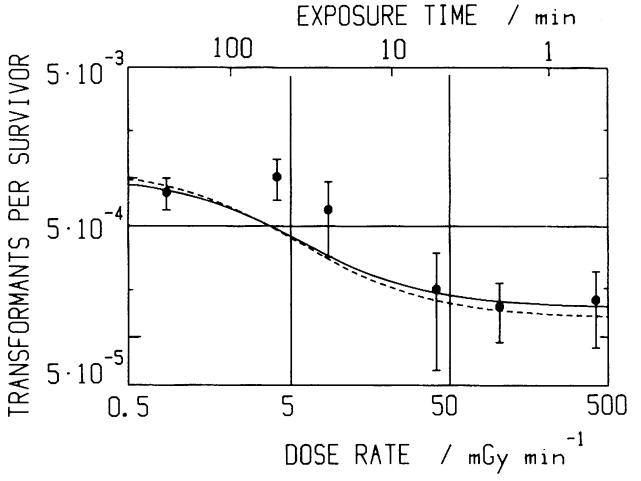

Figure 4. Dose rate dependence of neoplastic transformations of C_3H 10T1/2 cells for a dose of 210 mGy of fission neutrons according to data by Hill *et al.* (1984). The solid and the broken curve correspond to the assumption of a brief sensitive phase for transformations and to the parameters employed in the left panel (solid line) and in the right panel (broken line) of figure 3.

The estimated values of λ and τ can be tested against the transformation rates obtained by Hill *et al.* with 210 mGy of fission neutrons at various dose rates. The two curves in figure 4 correspond to the parameters utilized in the fits of figure 3. They are in general agreement with the data, although the comparison would suggest somewhat smaller values of τ.

It must be noted that the considerations apply only to the low doses and, as demonstrated by high dose data reported earlier (Hill *et al.* 1982), further complexities appear to arise due to the saturation at a yield of about 10^{-2} transformants per surviving cell and possibly also because of multi-event action.

In summary the surmise that C_3H 10T1/2 cells are especially sensitive to transformation during a brief period in the cell cycle appears to be to some extent supported by observations and provides a simple explanation for the dose rate effects observed with high-LET radiation. However the precision of the data available at this time is evidently inadequate to assert this explanation conclusively. Although other modes of action—such as the temporary induction of error-prone repair system (which would have quite different implications for the low dose extrapolation of the response)—cannot be excluded, it seems advisable to publish the hypothesis, if only because it may suggest various experimental approaches to the problem of cell transformation (and probably also that of carcinogenesis) by ionizing radiation.

Apart from obvious but technically difficult experiments at very low doses the validity of the hypothesis could be tested at higher doses by comparing the transformation frequency of cells that are exposed to neutrons at low dose rates while incubated under standard conditions as well as at a temperature low enough to stop progression through the cell cycle. In the latter condition the response should be the same as that obtained at high dose rate.

It is a striking aspect of this hypothesis that during the sensitive period a single high-LET event, in a gross sensitive volume that may be that of the nucleus, is very likely to produce the alteration which causes transformation; and that additional

events do not increase this probability substantially. A certain analogy to results for transformations of 3T3 cells by incorporated iodine-125 (LeMotte *et al.* 1982) may be noted. Equally surprising is the estimate of 5–10 min for the duration of this period. A possible explanation is that indirect action is involved. The irrelevance of the location of energy deposition may indicate that in a first step interaction between a high-LET particle and an ubiquitous component of the cell (probably water) a moderately short-lived molecule is produced which then interacts with a critical component at a critical time. This might be a structure that is involved in the control of an oncogene (e.g. a suppressor gene) and that is more accessible during replication. Because of the substantial distances involved, the radiolysis product is unlikely to be an active radical but it might be a peroxide. It should be noted that in this mechanism the sensitive period can be determined by the lifetime of the intermediate product rather than the duration of target sensitivity which could be even shorter than several minutes.

Finally, it should be pointed out that the situation should be still more complex in the case of low-LET radiations where, at least at higher doses, the dominance of multi-event action is probably indicated by the opposite dose-rate effect. This emphasizes the advantage of high-LET radiations in research in radiation oncogenesis (Rossi 1984).

References

BARENDSEN, G. W., 1985, Do fast neutrons at low dose rate enhance cell transformation *in vitro*? A basic problem of microdosimetry and interpretation (Letter to the editor). *International Journal of Radiation Biology*, **47**, 731–734.

BATEMAN, J. L., JOHNSON, H. A., BOND, V., and ROSSI, H. H., 1968, The dependence of RBE on the energy of fast neutrons for spermatogonia depletion in mice. *Radiation Research*, **35**, 86–101.

ELKIND, M. M., and HILL, C. K., 1985, Radiobiological issues in the anomalous enhanced effect of low-dose-rate fission-spectrum neutrons: reply to Letter to the editor by G. W. Barendsen (*Int. J. Radiat. Biol.*, 1985, **47**, 731). *International Journal of Radiation Biology*, **48**, 139–144.

GEARD, C. R., and HARDING, T., 1981, Morphometric studies with attached mouse C3H 10T1/2 Cells. *Annual Report of the Radiological Research Laboratory*, College of Physicians & Surgeons of Columbia University (Washington, D.C.: Department of Energy), pp. 274–280.

HALL, T. A., 1953, The interpretation of exponential dose-effect curves. *Bulletin of Mathematical Biophysics*, **15**, 43–47.

HILL, C. K., BUONAGURO, F. M., MYERS, C. P., HAN, A., and ELKIND, M. M., 1982, Fission-spectrum neutrons at reduced dose rates enhance neoplastic transformation. *Nature*, **298**, 67–69.

HILL, C. K., HAN, A., and ELKIND, M. M., 1984, Fission-spectrum neutrons at a low dose rate enhance neoplastic transformation in the linear, low dose region (0–10 cGy). *International Journal of Radiation Biology*, **46**, 11–15.

KELLERER, A. M., 1971, Considerations on the random traversal of convex bodies and solutions for general cylinders. *Radiation Research*, **47**, 359–376.

LEMOTTE, P. K., ADELSTEIN, S. J., and LITTLE, J. B., 1982, Malignant transformation induced by incorporated radionuclides in BALB/3T3 mouse embryo fibroblasts. *Proceedings of the National Academy of Sciences, U.S.A.*, **79**, 7763–7767.

ROSSI, H. H., 1984, Limitation and assessment in radiation protection. *Lauriston S. Taylor Lectures in Radiation Protection, Lecture No. 8* (Washington, D.C.: National Council on Radiation Protection and Measurements).

SINCLAIR, W. K., 1982, Fifty years of neutrons in biology and medicine: The comparative effects of neutrons on biological systems. *Radiation Protection*, Proceedings of the 8th Symposium on Microdosimetry, edited by J. Booz and H. G. Ebert (Luxembourg: CEC), EUR 8395EN, pp. 1–41.

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