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BIOLOGICAL IMPLICATIONS OF MICRODOSIMETRY: I. TEMPORAL ASPECTS*

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ABSTRACT: According to the theory of dual radiation action cellular injury by ionizing radiation is due to pairs of sublesions which in the case of low LET radiation are usually produced by statistically independent events due to separate particles (usually electrons). Therefore if low LET radiations are delivered at low dose rates repair processes can occur that may reverse sublesions before they have interacted with other sublesions, and the extent of such repair processes depends on the temporal distribution of absorbed dose. In contrast there is no influence of the temporal distribution of absorbed dose on that component of cellular damage which is due to the interaction of lesions produced in the same particle track, and which is dominant in the case of densely ionizing radiations.

Various aspects of the problem will be considered and theoretical interpretations of experimental results will be given.

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The basic postulate of the theory of dual radiation action (1), (2) states that the somatic effects of ionizing radiations on higher organisms are due to elementary lesions and that the yield, ϵ, of such lesions depends on the square of specific energy, z, in sites having a volume comparable to that of a cell nucleus. This leads to the basic equation:

$$\epsilon = k\overline{z^2} = k(\zeta D + D^2) \qquad \text{Eq. (1)}$$

where k can be considered to be a constant for purposes of this presentation. ζ is the dose mean of the distribution of z in single events* and D is the absorbed dose. The quadratic dependence implies that the lesions are produced by the interactions of pairs of sub-units that are produced at a rate that is proportional to z. We have referred to these sub-units as "impaired loci" and we have deduced that their production depends on energy concentration at a much smaller scale (probably of the order of nanometers). At this time there is little further that can be stated with any degree of certainty and the term "locus" should in particular not necessarily refer to a well-established physical entity. It seems quite possible, for instance, that a locus represents one or a group of molecules together with interaction or diffusion distances.

The right-most expression in Eq. (1) may be interpreted to the effect that the production of lesion can occur by two mechanisms, one, that is proportional to absorbed dose and represents interactions due to single events (or to single tracks provided this term includes not only the trajectories of primary particles but also those of its secondaries, e.g. delta rays), while the second term shich represents the square of the absorbed dose refers to lesions produced in unrelated events. It follows that the elementary lesions produced in the latter type of process can exhibit dose-rate dependence since the effects of the first event may be lessened or eliminated before the second event occurs. We shall give the

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*This quantity has also been denoted as $\bar{z}_D$ (3).

name recovery to this phenomenon with the understanding that this term may sometimes have been used in a different meaning. Recovery effects should be of particularly importance in the case of low LET radiations (e.g. x- and gamma radiation) where the quadratic term is usually dominant.

If one investigates the consequences of spatial differences of microscopic energy deposition, as for instance in LET experiments, one can assume that the only variable involved is the one under study and that in a well-conducted experiment the characteristics of the biological systems do not differ between the groups subjected to different radiation treatment. However, if temporal aspects of irradiation are studied as in dose-rate experiments, such an assumption is not necessarily correct because the characteristics of the biological system can change during the time interval over which the experiment is carried out. Thus, if synchronized cells are irradiated in a split-dose technique, the effectiveness of the second dose may vary considerably because progression of the cells around the cycle can substantially alter their radiosensitivity. Even in synchronous populations differential sensitivity can lead to partial synchrony after the first dose. This, together with such factors as division delay, can lead to a very complex response making it difficult or impossible to separate biological and physical factors in a dose-rate experiment. If the time over which a dose-rate experiment is carried out is sufficiently short so that the collective characteristics of the biological system have not altered appreciably, these factors are of course of little importance. However, it is difficult to establish whether this is indeed the case and any theoretical considerations which assume no change of the system under investigation have uncertain significance. However, if the theory is applied with this simplifying assumption to certain fractionation experiments, the results can be accounted for quite readily and it would seem that at least the principal features of other observations are also explained. This not only furnishes at least qualified support for the theory but also makes alternate explanations which invoke complex or hypothetical mechanisms inconclusive.

A general theoretical treatment of the influence of the temporal

distribution of absorbed dose has been given in another publication (2). Our purpose here is to present a few practical applications and to consider some extensions of the earlier treatment.

One subject that may deserve further consideration is that of recovery. The best known and most securely established form of recovery in higher organisms is that originally discovered by Elkind (4). It is characterized by a more or less exponential function with a characteristic period of the order of hours, and by apparent ultimate completeness. The effect is most readily observable - and was initially discovered - in mammalian cells in tissue culture where a few hours after irradiation surviving cells appear to respond to radiation in the same manner as cells that have never been irradiated. This effect is so well established and has so frequently been observed that there has been a tendency to consider it as the only recovery process of importance. However, neither characteristic of the process will invariably apply. Some cells, such as mature sperm, show little or no recovery even with very long intervals between fractions. Although evidence for intermediate levels of ultimate recovery may not be unequivocal, it would seem reasonable to assume that they exist. Indeed, even in Elkind's experiment one cannot exclude the possibility that recovery from sublethal injury is restricted to a level such as 80 or 90%. The existence of different characteristic recovery periods has also been established. Hornsey and coworkers (5) have demonstrated the existence of a repair process in intestinal crypt cells that takes place within minutes and Whitmore and colleagues (6) have shown that "potentially lethal lesions" are maximally repaired within 20 or 30 minutes. Evidence for much longer repair times that are comparable to or longer than the mitotic cycle may be uncertain, but this possibility cannot be excluded. The illustrative examples in our previous treatment of the problem which are based on complete recovery according to a simple exponential function must therefore be of limited applicability. However, this approach serves to clarify a number of basic issues and in particular misconceptions relating to dose-rate experiments.

Although we intend to restrict this discussion to elementary applications of theory, it is convenient to simplify the notation by expressing the absorbed dose in multiples of ζ. With

$$D = \Delta\zeta \qquad \text{and} \qquad c = k\zeta^2 \tag{2}$$

Eq. (1) becomes

$$\epsilon = c(\Delta + \Delta^2) \tag{3}$$

If Δ is given in N fractions, one may consider two extreme cases: the N doses are given in immediate succession, or the interval between successive fractions is so large that complete recovery has occurred. In the former case there is complete interaction between dose fractions and one obtains the effect given by Eq.(3); in the latter case there is no interaction between fractions and one merely obtains N times the effect of the dose Δ/N. Thus ϵ_N, the effect of the dose when given in N fractions, must lie between the following limits:

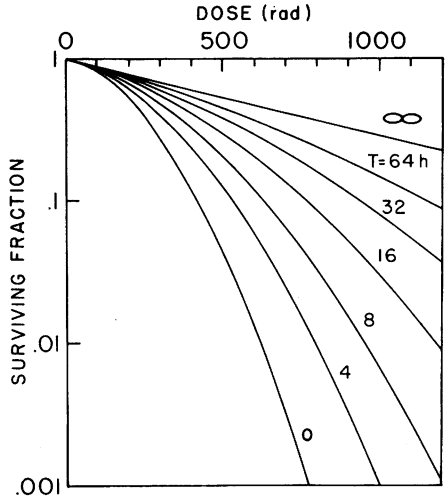
$$c(\Delta+\Delta^2) \geq \epsilon_N \geq Nc(\Delta/N + \Delta^2/N^2) = c(\Delta + \Delta^2/N) \tag{4}$$

It is evident from Eq.(4) that the effect of a given dose, Δ, decreases with increasing number of fractions provided the time interval between fractions is sufficient for complete recovery. In the usual case in which Δ is much larger than 1, it is thus possible to obtain very large differences in effectiveness provided N is large, i.e. at conditions approaching continuous irradiation at low dose rates. On the other hand, if N is 2, the effect cannot be reduced by more than a factor of 2.

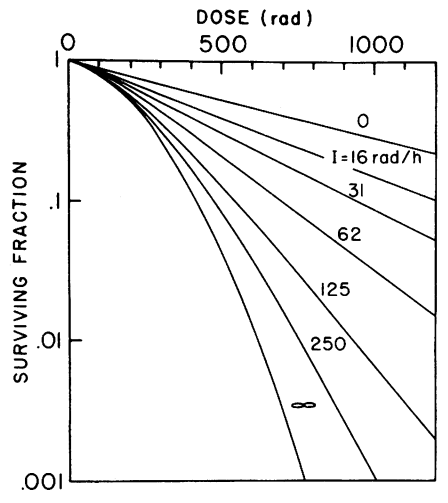
The situation is more complex when the time interval between fractions is insufficient for complete recovery. As we have shown (2) this requires integration of the expression

$$\epsilon = \int \tau(t)h(t)dt \tag{5}$$

where $\tau(t)$ is the recovery function and $h(t)$ the distribution of reparations of dose elements in time. Even with the simple assumption that $\tau(t)$ is an exponential, what appears to be a simple problem often becomes sufficiently complex to require solution by computer. Two examples of interest are illustrated in Fig. 1; they show hypothetical dose-effect curves following the simple (and probably somewhat simplistic) assumption



a



b

Fig. 1 - Dose-effect relation and its modification due to recovery

a) curves for constant dose rate, I.

b) curves for constant irradiation time T.

that the survival of a population of cells follows the relation

$$S = e^{-\epsilon} \qquad \text{or} \qquad \ln S = -\epsilon \tag{6}$$

This is equivalent to the assumption that each increment in the number of elementary lesions eliminates a proportionate fraction of the survivors.

Assuming a value $\zeta = 125$ rad (which is applicable for x rays in a site having a diameter of 1 micrometer) and setting $k = 10^{-5}$, one obtains curves which are quite similar to those obtained for mammalian cells in tissue culture. Curve 1a shows the results to be expected with employment of the usual technique in which the survival curves are obtained under conditions of constant dose rate. It should be noted that the survival curves are indistinguishable from straight lines before maximum recovery occurs at zero dose rate. When the dose rate is kept constant the period of irradiation increases linearly with dose; accordingly at large doses more recovery can occur during the irradiation period. If, on the other hand, in each group the time available for recovery during irradiation is kept the same by increasing the dose rate proportional to dose, the curves in Fig. 1b result. Here curvature is more evident even when the limiting case of infinite irradiation time is closely approached. The difference illustrated by curves 1a and 1b is of practical importance since it has been frequently assumed that a substantially linear survival curve obtained at some fixed dose rate indicates that the "one hit" component is the only one operative. It is evident that this conclusion can easily be erroneous.

The complexities of partial recovery may well be negligible in certain radiobiological experiments in which successive fractions are administered daily. In this case the same effect is produced by one fraction and by N fraction if

$$c(\Delta_1 + \Delta_1^2) = c(\Delta_N + \Delta_N^2/N) \tag{7}$$

This is equivalent to

$$\Delta_N/\Delta_1 = N/2\Delta_1 \left(\sqrt{1 + 4\Delta_1/N(1+\Delta_1)} - 1\right) \quad (8)$$

Fo r large values of N but a given value of Δ_1

$$\Delta_N/\Delta \sim 1+\Delta_1 \quad (9)$$

while in the limiting case of large values of Δ_1 one has

$$\Delta_N/\Delta_1 \sim \sqrt{N} \quad (10)$$

Δ_N/Δ_1 versus N is shown at the bottom of Fig.2 for various values of Δ_1. The top of Fig. 2 is based on a compilation by Phillips (7) of various recovery experiments. There is an obvious similarity between the two sets of curves and perhaps the most important aspect is that the limiting slope of one half (in these logarithmic plots) does not appear to be exceeded in the experimental data. Although the flatter experimental curves might be due to low values of Δ_1 (corresponding to a strong linear component) it should be stressed that similar curves are also obtained under conditions where recovery between exposures is incomplete because the tissues are only capable of limited recovery. On the other hand one obtains quite different curves if one assumes that the recovery is incomplete merely because the interval between successive fractions is not long enough, while there is no linear component and while the ultimate recovery is complete.

The theory can also be applied to the results of experiments in which a dose is given in two unequal fractions. We have shown (2) that in this case

$$\epsilon = c[\Delta+\Delta^2-2q\Delta^2\alpha\ (1-\alpha)] \quad (11)$$

where q varies between 0 and 1 and represents the degree of recovery during the period between the two irradiations, and α is the fraction of the dose given at the first irradiation. Eq.(11) indicates that a plot of ϵ versus α should be a parabola. The expression is symmetrical in α and $(1 - \alpha)$and reaches its minimum value when both of these quantities are equal to one-half. It will be noted that a parabolic shape is obtained regardlesscof the values of c, Δ or q, provided they are finite.

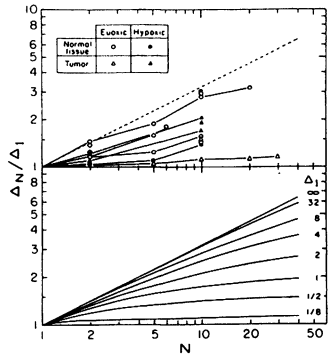


Fig. 2 - Δ_N/Δ_1 ratio of doses for equal effect in N fractions and one fraction, versus N. The curves in the upper quadrant are based on a compilation by Phillips (7).
The curves in the lower quadrant are derived from the theory of dual radiation action.

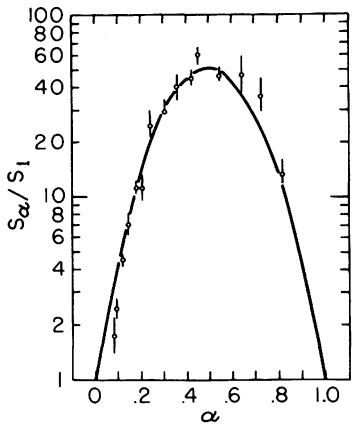


Fig. 3 - Irradiation of crypt cells with the dose given in two separated unequal fractions with the first fraction equal to α and the second equal to (1 - α). Data points by Withers (8). Curve based on the theory of dual radiation action.

Assuming again as in Eq.(6) that survival depends exponentially on one obtains

$$\ln S_\alpha/S_1 = 2q\Delta^2\alpha\lambda\ (1-\alpha) \qquad (12)$$

where S_1 is the survival if the dose is given in a single irradiation and S_α is the survival if the dose is split with the first fraction being equal to α. Fig. 3 shows the results of such an experiment in which Withers (8) irradiated jejunal crypt cells to a total dose of 1650 rads. It will be seen that the pattern experimentally obtained is basically that of a parabola, although the possibility of small but real differences in shape cannot be excluded.

Thus far the discussion has been concerned with two time periods. One is that which elapses between two interacting energy deposition events and the other is that required to erase the effect of the first of these events. It is, however, evident that there must be yet another characteristic time involved which is that required for interaction between two sublesions which according to the theory can be produced as far apart as a few micrometers. It would seem reasonable to assume that the sudden creation of the second sublesion does not immediately terminate the repair processes taking place at the other and that the reversal of either sublesion is possible until they interact with the result that the damage is fixed. Although the existence of such an interaction is a necessary consequence of the theory, there are few conclusions that can be drawn concerning its duration or its influence on the kinetics of the basic biophysical process. Perhaps the only statement that can be made is that it must be a rapid process at least in the dose-rate experiments described by Hornsey. If the interaction between sublesions required periods of the order of an hour it should be immaterial whether they are produced within seconds or within minutes of each other.

It may be of interest to speculate whether the potentially lethal lesions reported by Whitmore and collaborators are due to the fact that low temperature could perhaps delay the interaction of sublesions and thereby make more time available for Elkind type of repair which is known to be little affected by low temperature. Whitmore et al found the same phenomenon following the administration of drugs which interfere with RNA synthesis, but one might postulate that the interaction of lesions

requires a supply of RNA or for that matter any other key substance. However, while one can in this manner provide a qualitative explanation for the phenomenon of potentially lethal lesions, a quantitative explanation would have to rest on arbitrary assumptions regarding the incomplete reversal attainable. It would appear that depending on phase in the cell cycle only between 20 and 35% of the lesions can be eliminated by low temperature or drugs.

Since the development of potentially lethal lesions is determined by postirradiation treatment, one would assume that it is irrelevant whether they were produced in single events or pairs of events. Whitmore stated that these lesions are not found when cells are irradiated by high LET particles. He was, however, referring to heavy ions having an extremely high LET. It would seem reasonable that under such saturated conditions a 35% reduction of lesions would not influence lethality. Barendsen (9) has pointed out that potentially lethal lesions should be studied with radiations having a more moderate LET.

The recognition that the yield of sublesions must depend on the time interval required for the combination of the sublesion leads quite naturally to an evaluation of the initial separation of the latter. This is determined by spatial aspects which are the subject of the second paper.

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D I S C U S S I O N

Mr POWERS

I would like a clarification please. What does the function $S = e^{-k}$ describe biologically? Does it apply to single cell cultures for instance?

Mr ROSSI

It is probably a crude representation of a survival curve. As everybody here does or should know, a survival curve is a very complex thing. It involves populations in various stages of sensitivity, dynamics etc. However, basically one should expect survival to follow the shape of these curves. They represent the simplest assumption, namely that all cells have the same sensitivity i.e. the same K. Simple shapes are adequate to illustrate our point.

Mr POWERS

Yes, but what confused me was that at 250 rads per hour, and this means about 4 rads per minute, there is a very distinct curvature. I recall that there are innumerable curves of survival of mammalian cells which assume exponential behaviour at survival levels very much higher than depicted on the 250 rad/h curve. And the ordinary dose rates that are used are very much higher than 4 rads per minute.

Mr ROSSI

As I said, these curves are illustrative only and they can be shifted by a different choice of recovery parameter. I think these are based on a recovery time of about one hour or so.

Mr KELLERER

The example which has been chosen corresponds closely to the case of hamster cells irradiated in S-phase. In the experiment one can obtain curves with a somewhat shallower tail because it is difficult to remove the more sensitive cells completely.

Mr POWERS

But is it not true then in any experiment on survival curves, you try to isolate and control these things.

Mr ROSSI

We are merely trying to illustrate the difference between constant dose rate and constant time of delivery and we have used arbitrarily selected values in a simple model. The absolute numerical values are not meaningful.

Mr WIDERÖE

As you mentioned, there are three time intervals which are interesting. The first interval was the time when the cells were killed without any survivals. The second time interval was where you had rapid repair and the third time when the shape of the curve was recovered. Now I think that the two first intervals were investigated in some experiments by Berry in Oxford about two or three years ago. He gave very high doses in a very short time. Time was in the dimension of 10^{-8} sec or even less, and he found very interesting and very different survival curves from those obtained for smaller dose rates. I think that experiments similar to these could be used for investigating the phenomena you mentioned.

Mr ROSSI

I was talking about three somewhat different periods. The first time is the interval between the production of successive

lesions. The second period is the characteristic time of recovery of lesions and the third period begins after both lesions are produced and lasts until they combine.

Mr WIDERÖE

But at least I would mention that short time intervals have already been observed and used for experiments and I think that they could be evaluated.

Mr BARENDSEN

I have two questions. The first question concerns the graph in which you showed that the theoretical curve has a slope of 0.5. In comparing this slope with the slope of the curve derived from radiotherapy results, you said that it is satisfactory that these curves are not steeper than a slope of 0.5. I am interested in the fact that the actual slope is about 0.3. What is the corresponding value of zeta? What would be the implication of this slope with respect to the contribution of a single event type of damage?

Mr ROSSI

There are two possible explanations. The first is that there is a strong linear component. The other possibility is the one I tried to allude to earlier, namely that these cells are not capable of full recovery or at least not of the kind of recovery which Mr Elkind found. In fact some of the low curves are for testicular tissues which you would expect to be capable of only limited recovery.

Mr BARENDSEN

Can I ask another question please? You have mentioned the possible recovery of two sub-lesions which have to interact in order to cause lethal damage. Would you agree with the idea that such a sub-lesion might not be completely repaired, but might rather be rendered incapable of interacting with another lesion? In other

words the lesion might still be present for a long time and may still exert some influence, for instance in the effect that we see in culture as small colony formation. Thus, although the cell remains capable of unlimited proliferation, non-lethal damage is present and might impair the capacity for proliferation to a small but significant extent.

Mr ROSSI

Yes indeed; and I might add that under these conditions the cell may be much more sensitive to various chemical agents.

Mr BARENDSEN

That is also a possibility. In addition I would like to have your views about the possibility that the primary lesion or sub-lesion might be a complex type of damage, not caused by a single ionization, but by a number of ionizations in small volume.

Mr ROSSI

I would certainly agree that locus inactivation may be a complex process and that even the locus itself may not be clearly defined. It is likely to be a DNA molecule plus a diffusion distance.